HPV risk factors and screening among Malawian women

DISSERTATION

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Abstract

Introduction: Malawi has the highest age-adjusted incidence of cervical cancer in the world and a case-fatality rate of over 60%. Virtually all cases of cervical cancer are caused by the human papillomavirus (HPV). Most women will have an HPV infection during their lifetime but the majority will clear the infection without any symptoms or lasting impact. Several risk factors are associated with increased risk of progression from initial HPV infection to cervical cancer. Our study examined a potential risk factor for HPV infection and evaluated self-collection of samples for HPV testing. Specifically, we examined intravaginal practices (IVP) as a risk factor for HPV infection and abnormal cervical lesions among rural Malawian women. Second, we examined the validity and feasibility of self-collected sampling for HPV testing in a clinic setting. Last, we assessed the acceptability and identified correlates of acceptability for self-collection of samples for HPV testing in a community setting.

Methods: We used data from the baseline wave of a cohort study on sexual and reproductive health and a cross-sectional, clinic-based study examining sexual and reproductive tract infections. In our first aim, we used generalized linear models with a binomial distribution and log link and Fisher’s exact test to examine the associations between the type of intravaginal practices (IVP) (cleansing with soap and water, cleansing with cotton, cloth, or tissue, and inserting other substances) and frequency of...
IVP and HPV in 193 women presenting for care at a rural clinic in Lilongwe District, Malawi. Using this same population, we examined the feasibility, validity, and acceptability of self-collected sampling for HPV testing. In order to make these assessments we calculated a kappa statistic, sensitivity and specificity of self-collected samples compared to clinician-collected samples. Last, we used data from the baseline wave of a representative community-based cohort study among women of reproductive age (n=824) to examine correlates of willingness (developed based on the theory of planned behavior) to utilize self-collection of samples for HPV testing.

Results: In our first analysis, we found that intravaginal practices are common and frequently performed. When we examined the relationship between type and frequency of IVP with high-risk (hr) HPV or abnormal cervical lesions we did not find any significant associations. In our second analysis, when comparing self-collected to clinician-collected samples, we found high agreement in detection of hr-HPV (kappa 0.70-0.90) and high specificity (98%-100%) but varied sensitivity (50%-90%) by hr-HPV type. Last, in our third analysis, 67% of women reported being willing to self-collect a vaginal sample for HPV testing in their homes. Awareness of cervical cancer, supportive subjective norms, perceived behavioral control, and clinician recommendations were all positively associated with increased willingness to self-collect samples for HPV testing.

Conclusions: We did not find a significant association between IVP and hr-HPV or abnormal cervical lesions. However, our study population, and the high frequency with which IVP were reported, may have limited our ability to assess associations. We did find that self-collection in a clinic setting performed comparably to clinician-collected samples and care-seeking women found the procedure acceptable. Last, we found that
among a random sample of rural Malawian women, self-collection of samples for HPV testing is acceptable, and modifiable factors, like clinician recommendations, can help to facilitate the acceptability of the screening method.
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# Table of Contents

Abstract .................................................................................................................................................. ii

Acknowledgments .................................................................................................................................. v

Vita ...................................................................................................................................................... vii

List of Tables ....................................................................................................................................... xv

List of Figures ...................................................................................................................................... xvi

Chapter 1: Literature Review .............................................................................................................. 1

1.1 Background on HPV and cervical cancer ...................................................................................... 1

1.1.1 HPV and cervical cancer ........................................................................................................ 2

1.1.2 Risk factors for progression ..................................................................................................... 3

1.1.3 HPV and cervical cancer in Malawi ......................................................................................... 4

1.1.4 Screening ................................................................................................................................... 5

1.1.5 Cervical cancer screening in Malawi ....................................................................................... 7

1.2 Association between intravaginal practices and HPV ............................................................... 8

1.2.1 Key points .............................................................................................................................. 8

1.2.2 Purpose of intravaginal practices .......................................................................................... 8

1.2.3 Different types of intravaginal practices ............................................................................... 9

1.2.4 Frequency of intravaginal practices ....................................................................................... 10

1.2.5 Association between IVP and HPV ....................................................................................... 11
1.3 Validity and feasibility of self-collected samples for HPV testing .......................... 14
   1.3.1 Key points ........................................................................................................ 14
   1.3.2 Assessing validity of self-collected samples for HPV testing .......................... 14
   1.3.3 Factors associated with self-collection validity .............................................. 18
   1.3.4 Screening coverage with self-collected samples for HPV testing ................. 19
1.4 Acceptability of self-collection of samples for HPV testing ............................... 20
   1.4.1 Key points ........................................................................................................ 20
   1.4.2 Acceptability .................................................................................................... 21
   1.4.3 Barriers ........................................................................................................... 22
   1.4.4 Correlates of willingness to self-collect ....................................................... 22
   1.4.5 Theory of planned behavior ........................................................................... 23

Chapter 2: Parent study methods ............................................................................. 27

   2.1 Bwenzi la Thanzi (BLT) study methods ............................................................. 28
      2.1.2 BLT study setting .......................................................................................... 28
      2.1.3 BLT study population .................................................................................. 29
      2.1.4 BLT recruitment .......................................................................................... 30
      2.1.5 BLT sample size ......................................................................................... 30
      2.1.6 Data collection ............................................................................................ 30

   2.2 UTHA cohort study methods .............................................................................. 33
      2.2.1 UTHA study setting ..................................................................................... 33
      2.2.2 Study population ......................................................................................... 33
      2.2.3 Recruitment ................................................................................................. 34
4.3.3 Measures ................................................................................................................. 55
4.3.4 Analysis .................................................................................................................... 56
4.3.5 Ethical approval ....................................................................................................... 58
4.4 Results ......................................................................................................................... 58
  4.4.1 Participant characteristics ....................................................................................... 58
  4.4.2 Associations between IVP and hr-HPV ................................................................. 60
4.5 Discussion .................................................................................................................... 60

Chapter 5: Validity and acceptability of self-collected samples for HPV testing in rural Malawi: Findings from a clinic-based feasibility study ................................................. 68
  5.1 Abstract ..................................................................................................................... 68
  5.2 Introduction ............................................................................................................... 69
  5.3 Methods ..................................................................................................................... 71
    5.3.1 Study setting and population ............................................................................... 71
    5.3.2 Data collection ..................................................................................................... 72
    5.3.3 Data analysis ........................................................................................................ 73
    5.3.4 Ethical approval .................................................................................................... 74
  5.4 Results ......................................................................................................................... 75
    5.4.1 Study population and prevalence of hr-HPV infections with clinician-collected sampling .................................................................................................................... 75
    5.4.2 Agreement between clinician-collected and self-collected samples ............... 75
    5.4.3 VIA ....................................................................................................................... 76
    5.4.4 Acceptability ........................................................................................................ 77
5.5 Discussion.............................................................................................................. 77

Chapter 6: Factors influencing Malawian women’s willingness to self-collect samples for HPV testing.................................................................................................................... 88
6.1 Abstract .................................................................................................................. 88
6.2 Introduction .......................................................................................................... 89
6.3 Methods ................................................................................................................ 92
  6.3.1 Study design and population............................................................................ 92
  6.3.2 Measures ....................................................................................................... 92
  6.3.3 Statistical analysis ......................................................................................... 94
  6.3.4 Ethical approval ............................................................................................ 95
6.4 Results .................................................................................................................. 95
6.5 Discussion .......................................................................................................... 96

Chapter 7: Conclusions and implications for future research ................................. 104
7.1 Overview .............................................................................................................. 104
7.2. Aim 1 ............................................................................................................... 105
  7.2.1 Summary ..................................................................................................... 105
  7.2.2 Interpretation ............................................................................................... 105
  7.2.3 Public health significance ........................................................................... 105
  7.2.4 Future research directions ......................................................................... 106
7.3 Aim 2 ................................................................................................................. 106
  7.3.1 Summary ..................................................................................................... 106
  7.3.2 Interpretation ............................................................................................... 107
List of Tables

Table 1: Participant characteristics from the 650 sampled women in Malawi ............ 47
Table 2: IVP frequency, by individual practice of IVP among 650 women in Malawi. .. 50
Table 3: Participants motivations for IVP among 650 sampled women in Malawi ........ 51
Table 4: Participant characteristics of 193 care-seeking women in rural Malawi .......... 64
Table 5: Frequency and timing of IVP, by practice..................................................... 65
Table 6: Associations between frequency of IVP and hr-HPV ........................................ 67
Table 7: Unadjusted associations between IVP profile and hr-HPV ................................. 67
Table 8: Participant characteristics of 193 care-seeking women by clinician-collected
HPV results .................................................................................................................. 83
Table 9: HPV results, by collection modality................................................................. 84
Table 10: Concordance between HPV test results by self- and clinician-collected samples
........................................................................................................................................ 85
Table 11: Concordance between HPV test results by self- and clinician-collected samples
in women older than 30 years of age ............................................................................ 86
Table 12: Acceptability of self-collection\(^1\) .................................................................... 87
Table 13: Participant characteristics among 824 women sampled in Malawi ............ 101
Table 14: Attitudes towards cervical cancer and screening.......................................... 102
Table 15: Correlates of women's willingness to self-collect a vaginal sample for HPV testing

List of Figures

Figure 1: Progression from HPV infection to invasive cervical cancer ............................... 3
Figure 2: Theory of planned behavior .............................................................................. 24
Figure 3: UTHA research program .................................................................................. 27
Figure 4: Map of Malawi ................................................................................................. 28
Figure 5: Unadjusted prevalence ratios comparing different types of IVP and hr-HPV1. 66
Figure 6: Potential outcomes of GeneXpert HPV assay .................................................. 82
Chapter 1: Literature Review

1.1 Background on HPV and cervical cancer

Human papillomavirus (HPV) is the most common sexually transmitted infection among women worldwide. Most women will be infected with at least one type of HPV at some point during their lifetime.[1,2] HPV is a DNA virus and includes over 150 different genotypes. It is spread through skin or mucosal contact primarily during vaginal, oral and anal sex.[1,3] There are approximately 40 types of HPV responsible for genital tract infections and these can be classified as “low-risk” or “high-risk” HPV.[3] Low-risk (lr) HPV infections can sometimes cause warts, but are not cancerous. HPV 6 and 11 are the most prevalent lr-HPV types, causing 90% of cases of genital warts.[3] High-risk (hr) HPV is a causative agent for cervical cancer and is also associated with oropharyngeal, vulvar, vaginal, anal, and penile cancer.[3,4] Low-risk HPV infections typically clear slightly quicker at a median of 8.4 months while hr-HPV clears at a median of 9.3 months.[5] Some types are more oncogenic, such as HPV 16 and 18 which are associated with 70% of all cervical cancer cases worldwide.[6–8] The most prevalent types of HPV vary geographically. For example, in countries with higher overall HPV prevalence the proportion of HPV 16 is lower than in countries with lower HPV prevalence.[9] While most women become infected with HPV at some time in their lives, the majority will clear the infection within 1-2 years without any signs, symptoms or lasting impact.
Questions remain about risk factors for progression from HPV infection to cervical cancer and the best screening methods among populations at high risk for cervical cancer. Specifically, some important unanswered questions include the relationship of HPV to intravaginal practices, the willingness to self-collect samples for HPV testing in a community setting, and the validity, feasibility, and acceptability of self-collection of samples for HPV testing in a clinic setting.

1.1.1 HPV and cervical cancer

HPV is a necessary but not sufficient cause of cervical cancer. The progression from initial HPV infection to invasive cervical cancer takes an estimated 10-20 years from initial infection to tumor formation (Figure 1).[3,10] For most women, the initial HPV infection will regress to subclinical infection or clear within 1-2 years. However, for some women, the initial HPV infection can progress to a persistent infection. The persistent infection can either progress to cervical intraepithelial neoplasia or clear. Cervical intraepithelial neoplasia (CIN) is progressive from CIN 1 (mild dysplasia) to CIN 2 (moderate dysplasia) to CIN 3 (severe dysplasia and carcinoma in situ).[11] Finally, the precancerous lesion can either regress or turn into invasive cervical cancer. The virological mechanism of this progression from HPV infection to cervical cancer is not well understood, but the process has been illustrated by epidemiologic and laboratory evidence.[1] The natural history of cervical cancer makes screening for HPV and precancerous lesions a vital component of preventing progression from HPV to cancer.
1.1.2 Risk factors for progression

Many factors have been identified that put a woman at increased risk of progression from initial HPV infection to persistent HPV infection, cervical intraepithelial neoplasia or cervical cancer. Viral factors, environmental or exogenous factors, and host co-factors contribute to disease progression.[8] The type of HPV and location of infection are important factors in progression to cervical cancer. While infections occur equally in the cervix and vagina, cancer is mainly formed at the cervix.
transformation zone between the different kinds of epithelium in the genital tract.[1] Smoking, multi-parity and long-term use of oral contraceptives are all associated with increased risk of progression to cervical cancer.[1] Genetic factors and other host co-factors related to the immune response (like co-infection with other STIs) can affect risk of progression from the initial HPV infection to cervical dysplasia and cervical cancer.[8] HIV-infected women are more likely to have an HPV infection and persistence and are often infected with more types of HPV than HIV-negative women.[1] While several factors have been identified that increase risk of initial infection and progression from initial HPV infection, there is still uncertainty over what facilitates or prevents initial infection and then subsequent progression from HPV infection to cervical cancer. Therefore, further research is needed on factors that increase risk of initial HPV infection and progression to persistent infection, precancerous lesions, and cervical cancer.

1.1.3 HPV and cervical cancer in Malawi

Data on HPV prevalence in Malawi are limited. There are only two studies that measure HPV among Malawian women. The first study, from 1996, found that HPV was present in 23% of women without HIV and 60% of HIV-infected women.[12] However, the aim was to examine the role of HIV in modifying HPV incidence and it did not present overall prevalence of HPV or type-specific distribution.[12] A recent study of women in an HIV clinic in Lilongwe found that 58% of women were HPV positive while 39% of women had at least one high-risk subtype.[13] HPV 58 was the most prevalent high-risk type occurring in 9.5% of women.[13] As HPV is more likely to occur in
women with HIV this is most likely an overestimate of the prevalence in the general population. The WHO estimates that the HPV prevalence among women in eastern Africa is approximately 33%. In Malawi, this would mean over 1 million of the country’s 3.72 million women are estimated to be infected with HPV.[14] However, there are no countrywide data on the types of HPV most prevalent in Malawian women.

Cervical cancer is the most common cancer among Malawian women.[15] Malawi has the highest age-adjusted incidence rate of cervical cancer in the world. Malawi has an incidence rate of 76 cases per 100,000 women compared to 43 cases per 100,000 women in eastern Africa overall and only 14 cases per 100,000 women globally.[15] Among those who are diagnosed with cervical cancer in Malawi, over 60% will die from the disease.[4,14,15] With an estimated 2,314 deaths from cervical cancer every year, studies to explain Malawi’s distribution of HPV disease and risk factors for HPV infection are vitally needed.[14]

1.1.4 Screening

There are three standard screening tests used in secondary prevention of cervical cancer: cytology (also called Papanicolaou test, Pap test, Pap smear), visual inspection of the cervix with 3-5% acetic acid (VIA), and HPV DNA testing. In many countries, cytology is the standard screening test. In cytology, cervical cells are collected using a cervical swab and examined under a microscope by a cytopathologist.[11] While it has been successfully implemented and led to reduced cervical cancer incidence in many countries, cytology has been hard to establish as a screening program in many developing countries. If available at all, screening facilities are often only located in
urban areas.[16] Additionally, the results of the test are not available at the same visit, thus adding the burden of follow-up visits.[16] Cytology also requires a laboratory with highly trained personnel to prepare, stain and read the slides, which is not available in many regions.[17]

In many settings without access to laboratory services or a cytopathologist to examine cervical swabs, VIA is used to screen for cervical dysplasia. VIA involves unaided (naked eye) visual inspection of the cervix after application of 3-5% acetic acid. With application of acetic acid, abnormal tissue is identified by acetowhite areas in the transformation zone, close to the squamocolumnar junction or cervical os.[11] VIA is a low-tech and easily implemented test in settings where more complex laboratory procedures are not possible. A 2009 study funded by the NIH validated VIA as an acceptable screening method for cervical cancer in Kenya.[18] However, other studies, including a population-based study of a VIA program in Nigeria, found that there was high variability by provider in the quality of VIA screening.[16,19,20] In general, studies have found that VIA has high specificity (89% and 98% respectively) but poor sensitivity (32%[17] and 47% [19] respectively) compared to cytology and HPV testing.[17,19]

HPV DNA testing is used as a secondary screening test in conjunction with Pap testing or even a first line screening method in certain settings. HPV testing assesses for presence of an HPV infection using cervical or vaginal samples. The WHO recommends hr-HPV DNA testing as the primary cervical cancer screening in places where cytology has not been established. Studies have consistently shown that HPV testing has higher sensitivity (80% vs. 71%) but lower specificity (57% vs. 72%) than cytology in
There are currently four FDA approved hr-HPV tests that use either hybrid capture or polymerase chain reaction (PCR). The samples for HPV testing can be taken by a clinician or taken as a self-collected sample either in the clinic or in an alternative setting.

Regular screening for HPV and CIN, treatment of precancerous or cancerous cervical lesions through cryotherapy and loop electrosurgical excision procedure (LEEP), and primary prevention through HPV vaccination have resulted in dramatic reductions in cervical cancer incidence in many countries. However, screening programs in eastern Africa are limited due to lack of health delivery infrastructure and trained personnel, limited health budgets and competing healthcare priorities. For women who are considered at high risk of HPV infection or those who cannot be reached for regular screening, self-collection of a vaginal sample for HPV testing has successfully been used in sites around the world to increase screening. However, before a successful screening program can be established, it is necessary to know the feasibility and barriers to implementing a screening program.

1.1.5 Cervical cancer screening in Malawi

Malawi first implemented a national cervical cancer screening program using cytology in the late 1980s. However, the program quickly deteriorated due to lack of financing, trained clinical providers, and technical capacity in the laboratory. A pilot screening program using VIA was launched in 8 clinics in southern Malawi in 1999 and has since expanded to 81 health clinics as of 2011. Despite the expansion, the screening programs still have many obstacles and most Malawian women have never
received screening. In two qualitative studies of VIA providers in 13 [36] and 14[38] districts in Malawi several obstacles were listed as barriers to providing care, including: shortage of supplies to deliver VIA (such as shortage of acetic acid), the need for referrals to sometimes distant central hospitals for treatment, shortage of staff, and misconceptions about VIA from women and their partners.[36] Encouragingly, when women do come in for cervical cancer screening they were satisfied with services. In exit interviews from a VIA clinic 112 women reported being satisfied with services and 68% reported that they were very satisfied.[39]

1.2 Association between intravaginal practices and HPV

1.2.1 Key points

- Women commonly perform intravaginal practices (IVPs) in many parts of the world.
- Intravaginal practices comprise a broad category of substances and application methods, which vary in frequency of use and timing, and serve various purposes.
- Limited research suggests an association between intravaginal practices and increased HPV infection and abnormal cytology with mixed findings on the direction of the association.

1.2.2 Purpose of intravaginal practices

Intravaginal practices encompass a wide range of substances and application methods, such as cleansing with soap and water or inserting cotton, cloth or tissue,
which vary in frequency of timing and use. Women use intravaginal practices for a variety of purposes related to maintaining genital health, personal hygiene and sexuality.[40] Women douche (flush out the vagina by inserting pressurized liquid) for hygiene or to prevent infection.[41–43] Among rural women in Laos with limited knowledge of cervical cancer, 70% suggested frequent vaginal douching as a method of cervical cancer prevention.[44] Other studies have shown that women believe that cervical cancer results from poor vaginal cleansing.[45,46]

**Women also use intravaginal practices to dry or tighten the vagina.** In many cultures women view a dry vagina as indication that a woman has not had sex with another man. IVP are also used for sexual pleasure for both the male and female partner, depending on the cultural context.[47] Women also associate dryness with a healthy vagina as many common vaginal infections are associated with discharge or wetness.[44,47]

1.2.3 Different types of intravaginal practices

**Women use different types of intravaginal practices and substances for different purposes.** In the US, women use both products that are available commercially and some that can be created from household items like yogurt, bleach or vinegar.[48] Participants in a four-country study in Asia and Africa, most often reported using substances like herbs, bath soap, and lemon.[43] In Zimbabwe and Malawi, women used a wide range of substances and application methods depending on the purpose of the vaginal practice. Women inserted or ingested herbal or other natural substances to dry and tighten the vagina; they inserted cloth or exposed the vagina to air or heat to remove
vaginal secretions; they finger-cleansed using water or detergent-like products for cleaning; they used cooking oil or Vaseline intravaginally to lubricate; they swallowed or inserted tablets such as aspirin to reduce pain; and they consumed or inserted sugary products to dry the vagina and make it “sweet.”[47] The type of substance varies by country and within countries by region or ethnic group.

Many women who perform vaginal practices will also use multiple substances at different times. In Mozambique, women performed multiple types of intravaginal practices for different reasons. In the study, 92% of women practiced intravaginal cleansing and of these women 94% did it for hygiene and to increase cleanliness.[43] Seventy-two percent of these same women also inserted dry substances intravaginally, with 87% doing so to create a dry vagina that had the ultimate goal of increasing male sexual pleasure.

1.2.4 Frequency of intravaginal practices

The frequency of intravaginal practices can vary by type and purpose of the practice. Some women only use certain practices, like vaginal douching, sporadically, such as once a month.[48] These same women may use other practices, like inserting cloth, multiple times daily.[43] As with the type of vaginal practice performed, the frequency with which women perform given intravaginal practices depends on a combination of cultural aspects, the type of practice and the intended purpose of the IVP. Intravaginal practices vary by the specific substances used, the method of application and the frequency of the practice.
1.2.5 Association between IVP and HPV

The association between vaginal practices and abnormal cytology was suggested as early as the 1930s.[49] While IVPs are associated with certain adverse health events like pelvic inflammatory disease, ectopic pregnancy, HIV, bacterial vaginosis and other sexually transmitted infections [48], there are mixed findings on whether intravaginal practices have a protective or harmful association with HPV infection and abnormal cytology.[42,48,50,51]

IVP as a protective factor for abnormal cytology. Some studies show that regular use of vaginal practices, in particular douching, can be protective against HPV infection and cervical cancer. In a cross-sectional study of FSW in Burkina Faso, regular douching was associated with a 75% reduction in the odds of detection of lr-HPV subtype 6 or 11.[52] However, douching was also associated with a non-statistically significant increased odds for all types of HPV (including high-risk types).[52] A case-control study found a 17-fold increased odds (95% CI: 4.2, 74.7) of cervical cancer among those who had never used any vaginal practices. However, this estimate was based on only 3 out of 82 cases who had ever vaginally douchet and the imprecision is reflected in the very wide confidence interval.[53] Last, an in vitro study conducted in Taiwan also provided evidence that douching may protect against HPV infection.[54] The researchers found that simulating vaginal douching by cell washing cervicovaginal secretions that had been exposed to HPV reduced the infectious load by 82-93%.[54]

Potential protective mechanisms. Some studies suggest that regular vaginal douching removes HPV from the place of infection and thus shortens the duration of viral
Another explanation for the protective association between IVP and abnormal cytology is that what is viewed as a protective association is really just a reflection of decreased DNA detection after vaginal douching, not decreased infection with HPV. However, other research suggest that HPV detection is actually increased after douching with 5% acetic acid.

**IVP as a risk factor for abnormal cytology.** Several studies illustrate an association between IVP and increased odds of HPV or abnormal cytology. To examine associations of IVP with increased prevalence of HPV or cervical carcinoma, it is necessary to look at association by type and frequency of practice.

**Type of IVP.** Many studies present the association with douching using various substances. In the study of FSW in Nigeria, recent douching with lemon or lime juice was associated with a 76% increased odds of cervical dysplasia after controlling for HIV status (95% CI: 1.00, 3.10). In a study of low-risk women in Utah, there was not a significant difference in association between different douching preparations (chemical, commercial, water/vinegar, water/soda, water only) and cervical carcinoma. In another US-based study among urban adolescents, women who douched in the past 90 days had a 2-fold increased odds of any type of HPV after adjusting for lifetime number of sex partners and age difference between partners. In one review, women who used commercial preparations for douching had 2.4 times the odds of cervical cancer as women who never douched, but use of water or vinegar only did not lead to an increased odds of cervical cancer compared to women who never douched. Most studies examining the type of substance used for IVP compare women reporting use of one substance to women not performing any practice or only using water and are unable
to compare risks of different practices. Therefore, studies are needed that compare different types of application methods and substances in the same population.

*Frequency of IVP.* While many studies only show borderline association between type of IVP and cervical abnormalities, the relationship between greater frequency of IVP and abnormal cytology is stronger.[42,48,56,57] The study of 408 women in a low-risk population in Utah found that douching more than 4 times per month increased the odds of abnormal cytology by 4.7 times, compared to those who never douched after adjusting for age, lifetime number of sex partners, cigarette smoking, religious activity and education. They did not find a significant association between those who douched less frequently compared to those who never douched.[56] A meta-analysis found that there was a weak overall association between ever douching and abnormal cytology, but among women who douched at least once a week, the pooled adjusted relative risk was 1.86 (95% CI: 1.29, 2.68).[42] Another review article found that among seven studies that measured frequency of douching (with “frequent” ranging from >2 times/week to >4 times/month) there was an increased odds of cervical cancer for more frequent practices in all studies, although only four of the seven were statistically significant associations.[48] Last, among a sample of adolescent Tanzanian girls IVP was associated with 2.19 times increased odds of HPV (95% CI, 1.09–4.39) compared to those who did not report IVP. They also found a dose-response relationship with frequency of IVP and HPV.[58]

*Possible mechanisms for IVP as risk factor for abnormal cytology.* For both HPV infection and other STIs, intravaginal practices may increase susceptibility of infection because of alterations in the vaginal pH, microflora or cervical mucosa.[56,57,59] The
plausibility of this mechanism is supported by studies that illustrate more frequent IVP was associated with higher prevalence of cervical carcinoma as those women have a continual disruption of the normal vaginal environment.[56] Similar to the mechanisms for a protective association, IVP may not affect the actual infection, just detection. Douching or other IVP can induce cervical friability (making the cervix easily irritated and prone to bleeding) which increases the cellular yield of cervical samples and may increase detection of HPV.[57]

1.3 Validity and feasibility of self-collected samples for HPV testing

1.3.1 Key points

- In general, self-collected vaginal samples for HPV testing have high concordance and similar sensitivity and specificity to clinician-collected cervical samples.
- Several factors can influence the validity of self-collected samples for HPV testing, include the type of collection method (ie vaginal swab vs. lavage), population under study, and type of HPV testing (hybrid capture vs. PCR).
- Self-collected samples for HPV testing is an effective way to increase cervical cancer screening rates and reach under-served and hard-to-reach populations.

1.3.2 Assessing validity of self-collected samples for HPV testing

Compared to other cervical cancer screening methods, HPV testing examines the initial HPV infection rather than development of abnormal cervical lesions. HPV testing can be performed on clinician-collected cervical samples or vaginal or cervical self-
collected samples. A cervical sample is taken from the cervix using an endocervical broom or brush/spatula combination and a vaginal sample can be taken from anywhere in the vagina using a swab. While there are no guidelines for what qualifies as good sensitivity and specificity for cervical cancer screening, cytology has a sensitivity of 70-80% and specificity of about 95% for identifying CIN 2-3 compared to colposcopy.[3] Validity of self-collected samples can also be measured by calculating a kappa statistic comparing expected to observed agreement by overall hr-HPV or by subtype of hr-HPV. As a general guideline, a kappa statistic between 0 and 0.40 is considered poor agreement; 0.41-0.75 is intermediate to good agreement; and >0.75 is excellent agreement beyond chance.[60,61]

Clinic based studies. As early as 1993, evidence supported good agreement between self- and clinician-collected samples for HPV testing with 91% concordance between the two tests.[62] Since this study, several meta-analyses have compared self- and clinician-collected sampling. In 2005, a meta-analysis of 12 studies calculated a pooled sensitivity of 0.74 (95% CI: 0.61, 0.84) and specificity of 0.88 (95% CI: 0.83, 0.92) comparing self-collected to clinician-collected samples.[63] A 2007 meta-analysis of 18 studies found good agreement between the two sampling methods with a kappa (κ) of 0.66 (95% CI: 0.56, 0.76) although they noted that there was a higher prevalence of low risk HPV types in self-collected samples.[64] Another 2007 systematic review of 25 studies found significant variation with kappa values ranging from 0.24 to 0.96.[24] An updated review from 2011 of 19 studies also found high agreement with a κ=0.81 (95% CI: 0.64, 0.96) for sampling with a swab using PCR for testing.[65] They also found that
agreement varied with the sampling device (swab, brush, lavage) and detection method (hybrid capture or PCR).[65] In all of the reviews, the authors note that there was heterogeneity in the estimates due to different populations (screening or referral), methods of collection (swab, lavage, tampon), testing method (PCR or hybrid capture), and age and geographical location of participants.

Clinic-based studies in low resource settings. While providing evidence that self-collected vaginal samples for HPV tests can perform comparably to clinician-collected cervical samples, the majority of the studies included in the meta-analyses were US-based or in settings with established cervical cancer screening programs. More recently, studies on self-collecting samples for HPV testing have been expanded to low resource settings where women are not able to access other cervical cancer screening. In a study of 8,556 women in rural China there was not a difference in sensitivity or specificity for CIN 3+ (using cytology as the gold standard) between clinician- and self-collected samples.[66] In a multi-country study of a new low-cost HPV test (careHPV) in 20,461 women in Nicaragua, India, and Uganda, clinician-collected samples had the highest sensitivity (81.5% for CIN 2+ and 85.3% for CIN 3+) and highest specificity (91.6% for CIN2+ and 91% for CIN 3+) compared to self-collected samples, VIA and cytology.[67] Self-collected vaginal samples had the next highest sensitivity and specificity and performed better than VIA and cytology in identifying precancerous lesions.[67] In contrast, a multi-center cross-sectional study of 334 women in Tucson, Arizona; Hermosillo, Mexico; and Lima, Peru found that sensitivity was significantly lower for self-collected samples compared to clinician-collected samples (49% for self-collected vs. 82% for clinician-collected) although specificity did not significantly differ in ruling
Overall they calculated $\kappa = 0.55$ (95% CI: 0.43, 0.61) with an overall concordance of 77% between the two sampling methods.[68] A South African study of 1,415 unscreened women comparing self-collected vaginal samples with clinician-collected cervical sample, Pap smear and VIA found that the HPV tests were less specific than Pap smears but more sensitive for CIN 2+.[69] This study found a lower agreement between self and clinician-collected samples ($\kappa = 0.45$).[69] Another study among 450 South African women found much higher agreement ($\kappa = 0.73$).[70] Compared to the clinician-collected samples, the vaginal swab samples with PCR testing had a sensitivity of 0.82 (95% CI: 0.67, 0.93) and a specificity of 0.88 (95% CI: 0.76, 0.96).[71] A third South African study among 15 adolescents found high agreement for hr-HPV ($\kappa = 0.73$) and moderate agreement for lr-HPV ($\kappa = 0.59$).[72] A clinic based study in rural Gambia of 377 women found that self-collected swabs had a sensitivity of 0.64 (95% CI: 0.52, 0.83) and specificity of 0.94 compared to collected cervical samples.[73] In a study comparing self- and clinician-collected HPV samples in 606 women in Uganda, the agreement for any hr-HPV type was high ($\kappa = 0.87$; 95% CI: 0.82, 0.92) and type specific agreement ranged from a low of $\kappa = 0.41$ for HPV 70 to $\kappa = 1.00$ for IS-39.[74] Similar to the US-based studies, comparisons between self- and clinician-collected samples in lower resource settings were heterogeneous although most found moderate agreement between the two sampling methods.

Community based studies. While the majority of studies comparing self- and clinician-collected samples were done in a clinic setting, there are a few studies that explored community based sampling. One US-based study compared clinician-collected
samples to self-collected cervicovaginal specimens collected at home and mailed in for testing among 443 women in the Mississippi Delta region.[75] No significant difference was found in HPV detection between clinician and self-collected samples.[75] Using three different HPV DNA assays, kappa values ranged from 0.602 to 0.612 comparing clinician-collected cervicovaginal samples to participant self-collected cervicovaginal samples.[75] Another study among 878 women in Brazil used community health agents to pick up home-based self-collected vaginal samples for HPV testing and compared them to samples collected by the clinician in the healthcare facility. The agreement between the self- and clinician-collected samples was high (κ=0.70) and there was similar sensitivity and specificity for identifying high and low grade lesions.[76] While research is limited on non-clinic based studies, the current evidence suggests that study setting does not affect self-sampling validity.

1.3.3 Factors associated with self-collection validity

Much of the heterogeneity in comparisons between studies can be explained by differences in study design. HPV prevalence varies with age, with more infections under age 30. Several studies compared self-collection in different age groups. A South African study of 16-17 year olds found that self-collection was comparable to clinician-collected samples (κ=0.80, p<0.001).[77] Another South African study found there was not a significant difference in test by age, although the population only included women aged 35-65.[69] Last, the meta-analysis of 18 studies found that the HPV detection rate between self- and clinician- collected samples did not vary across age groups.[64]
Another concern with the validity of self-sampling is the difference in sampling location (vaginal vs. cervical samples). Many studies showed that detection was comparable for hr-HPV types, but lr-HPV types were more often identified in vaginal specimens.\[64,69,72,78,79\] In a study of 332 women with HPV and 66 women without HPV, cervical (similar to clinician-collected) and vaginal samples (similar to self-collected) were compared for forty different individual HPV types. They found that lr-HPV types were more prevalent in vaginal specimens than cervical specimens, but that there was not a statistically significant difference in detection of hr-HPV types.\[78\]

1.3.4 Screening coverage with self-collected samples for HPV testing

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. A meta-analysis of 10 studies in North America and Europe found that compliance with HPV self-collection was significantly greater than with cytology (RR 2.1; 95% CI: 1.30, 3.52).\[80\] Another review of 28 studies found that in countries with established screening programs, self-collection is more effective than sending out a reminder for cytology in increasing the screening response rate among underscreened women.\[25\] In two studies conducted in Sweden, HPV self-sampling kits mailed to the women’s homes were moderately successful for increasing screening in women who did not come in for care.\[81,82\] A study among 2,480 Italian women found that directly mailing self-sampling kits compared to letters inviting women to clinic-based cytology or HPV testing, increased compliance by 41% (95% CI: 1.10, 1.82).\[83\] In a randomized control trial (RCT) of under-screened women in Argentina, women who were offered
self-collection in the home through community health workers were 4 times more likely to be screened for cervical cancer (95% CI: 3.44, 4.71).[35] Among women who had not had a Pap test in the past four years, those who were offered self-collection were 5 times more likely to be screened than women in the control group (RR 4.98; 95% CI; 4.12, 6.02).[35] In another RCT 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 cancer screening.[84]

In a small exploratory study of underserved women in rural Appalachia, self-collected HPV testing was offered to women overdue for cervical cancer screening. Among the 31 women approached, all participated and 90% of women with positive results received subsequent cytology.[85] In a feasibility study of self-sampling among 296 homeless women in downtown Vancouver, all of the participants provided a self-collected specimen and 82% of women who tested positive for HPV were recontacted for follow-up.[86] Finally, a study of 26,409 women in the Netherlands found that not only were women in the mailed self-sampling arm more likely to respond than the control arm (30.8% for self-collection vs. 6.5% in control arm invitation for cytology), p<0.001), but among those who were HPV+, 89% followed up for cytology triage.[87]

1.4 Acceptability of self-collection of samples for HPV testing

1.4.1 Key points

- In many settings, women have found self-collection of samples for HPV testing acceptable and easy to perform.
- Acceptability of self-collection of samples for HPV testing in non-US settings and countries without established cervical cancer screening programs is less known.
- The theory of planned behavior provides a framework to examine attitudes and beliefs about self-collection for HPV testing.

1.4.2 Acceptability

While there are many studies from countries around the world examining the validity and reliability of self-collected samples for HPV testing, the research on the acceptability of self-collected samples for HPV testing, especially in non-US settings, is limited. Available findings suggest that most women are willing to self-collect and find self-collection easy to perform, less embarrassing, and less painful than a pelvic examination.[21,33,88–97] However, in comparisons between HPV DNA testing using self- or clinician-collection and the standard pelvic exam there are mixed findings on women’s preferred method.[21,32,33,88,89,93–96,98] The method in which the self-collection program is implemented can increase acceptability. Past research identified some methods as effective in increasing acceptability, such as having a provider in the clinic room and using detailed pictures, dolls or models to illustrate how to self-collect.[91]

Successful implementation of self-collection of samples for an HPV screening program will also require provider approval.[91] Studies have shown that clinicians support including HPV testing (including both self- and clinician-collection) as part of their screening program. In one study in Thailand, providers were most in favor of a combination screening program where women self-collected samples in villages and
women with positive tests would come into the clinic for VIA screening.[99] Clinicians were most in favor of this option as it was proactive in bringing screening to the women rather than requiring them to travel far distances to the clinic.[99]

1.4.3 Barriers

In past studies, women who self-collected samples for HPV testing reported willingness to do so again in the future. Many of these same women also reported that they preferred when clinicians collected the sample. The top concern among women was the fear they were not getting a good enough sample when self-collecting and that the test was not as reliable as when the sample was collected by a clinician.[24,33,89,91–94,98] Women also preferred clinician-collected samples as they valued seeing the clinician.[88]

Other barriers include concerns about privacy, pain or injury. [24,33,91] Some studies show that women in regions where vaginal practices are common could experience more pain due to excessive vaginal dryness.[24,96] In countries with established cytology screening programs women may be less willing to switch to self- or clinician-collected HPV DNA tests as they are already familiar with the other screening methods.[98]

1.4.4 Correlates of willingness to self-collect

Before a screening program using self-collected HPV testing can be successfully implemented, it is beneficial to understand the characteristics of women least willing to self-collect samples for HPV testing and thus better develop
interventions to target these women. Past research in the US, Nicaragua and Mexico found no sociodemographic variables associated with willingness to use a self-test.[94,96,97] One US-based study of sexual minority youths found that older age was associated with willingness to self-test.[100] Reproductive history as well as menopausal status, current tampon use or ever use of home pregnancy kit also were not significant predictors of willingness to self-collect a sample for HPV testing.[88,97] Other studies have found that low education and low knowledge of cervical cancer and HPV are associated with a lower willingness to self-collect.[24,32,89] In order to assess the association between beliefs, attitudes, knowledge and willingness to self-collect for HPV testing, we will use the theory of planned behavior.

1.4.5 Theory of planned behavior

The theory of planned behavior (TPB) is a theory of reasoned action that incorporates both personal factors and social influences in predicting the intention to perform a particular behavior.[101] TPB is based on the premise that behavioral intention is the most important determinant of behavior.[102] It is comprised of three conceptual constructs: attitudes, social norms and perceived behavioral control (Figure 2).[102,103] Depending on the behavior, one of the constructs may be a better predictor of intention or a construct may not be relevant at all for certain behaviors.[103]
**Attitude towards behavior.** The attitude towards the behavior is **influenced by both behavioral beliefs and evaluation of behavioral outcomes.**[102] The attitude construct represents the personal factor in the model where the individual’s positive or negative evaluation of performing the behavior contributes to their intention to perform the behavior.[101] Attitude towards the behavior includes both beliefs about what is required in performing the behavior and the potential outcome that will result if they perform the behavior.[102] In this construct, if a person views the behavior as providing
favorable consequences they will have a favorable attitude and be more likely to perform the behavior. For example, for self-collection of samples for HPV testing in the home, the attitude toward the behavior component would take into account a woman’s concern about her perceived risk and severity of cervical cancer and also her belief about whether or not screening would improve her health.

Subjective norm. The subjective norm construct reflects the perceived social pressure to perform or not perform the behavior. Both normative beliefs and motivation to comply can influence the subjective norm construct. Normative beliefs are those beliefs that an important individual or group approves or disapproves of performing the behavior. For cervical cancer screening, the subjective norm construct would consider whether a woman feels people who are important to her (such as her partner or her clinician) would want her to get screened.

Perceived behavioral control. Perceived behavioral control was added onto the first two constructs in the theory of reasoned action to create the TPB. This last construct is a reflection that attitudes and social norms do not matter if the person does not believe that the behavior is under her control. This construct refers to the perceived ability to perform the behavior. For example, this would assess whether a woman feels she could self-collect a sample for HPV testing correctly and take into consideration those potential barriers she feels might hinder her ability to do so.

Limitations of the TPB. In the TPB model, intentions are assumed to reflect the motivational factors that influence a behavior. In practice, the questions used to measure
intention may not be an adequate measurement of these factors.[104] A meta-analysis of
161 studies using TPB found that the theory is stronger in predicting intention (R² = 39%)
and self-reported behavior (R² = 27%), but is less reliable in predicting actual observed
behavior (R² = .20).[104]

There also are limitations with regards to the incorporation of subjective norms as
many studies find it to be a less reliable factor for intention to perform a behavior than
other constructs.[104] However, others argue that some research used single item
measures for TPB constructs instead of more reliable multi-item scales.[104]

TPB in attitudes towards cervical cancer screening. The TPB has been used in
several studies to examine intentions around cervical cancer screening. In one study of
142 women in London, the TPB explained a significant proportion of variation in
attitudes towards the Pap test.[105] Among Latina women in the United States, perceived
behavioral control and attitude were highly correlated with intention to have a Pap
test.[106] In another US-based study, attitude was one of the major determinants of
intention to obtain a Pap test while subjective norm was not significantly associated with
intention.[107] Among Canadian women, attitudes (OR 1.22; 95% CI: 1.15, 1.30)
indirect subjective norms (OR 1.02; 95% CI: 1.01, 1.03) and perceived behavioral control
(OR 1.16; 95% CI: 1.10, 1.22) were all significantly associated with intention to undergo
HPV testing.[108] While the TPB is used to assess intention to obtain a Pap test, only one
study applied the framework to HPV testing. To our knowledge, our study is among
the first to use TPB to assess intention to self-collect a sample for HPV testing and
the first to use the TPB in a population without an established cervical cancer
screening program.
Chapter 2: Parent study methods

This dissertation project is nested within two studies at the research site at the McGuire Wellness Center of Child Legacy International (CLI) in Mkango, Malawi. Aims 1 and 2 were part of a clinic-based study, Bwenzi la Thanzi (“BLT”) examining sexually transmitted and reproductive tract infections while Aim 3 was part of the Umoyo wa Thanzi (“UTHA”) cohort study investigating the role of decision making in reproductive health (Figure 3).

Figure 3: UTHA research program
2.1 Bwenzi la Thanzi (BLT) study methods

2.1.2 BLT study setting

*Health Clinic.* This study was conducted at CLI’s McGuire Wellness Center. The McGuire Wellness Center is a rural clinic that opened in June 2012 in Lilongwe District, Malawi (Figure 4). It currently offers outpatient services such as childhood immunizations, family planning services and basic treatment for genitourinary, respiratory, gastrointestinal and other communicable and non-communicable diseases. The center also has trained HIV counselors who offer voluntary HIV counseling and testing. The McGuire Wellness Center’s catchment area covers 68 villages (~20,000 people).

Figure 4: Map of Malawi
Laboratories. The McGuire Wellness Center has an on-site basic laboratory with a refrigerator, -20°C freezer, centrifuge, ELISA plate reader, and light microscope. The majority of laboratory testing for the BLT Study was conducted at the UNC Project laboratory in Lilongwe. UNC Project lab supports local studies conducted by UNC Project, a large HIV clinic, and other international studies in collaboration with partners. The UNC Project lab is the first diagnostic pathology laboratory in Lilongwe and is accredited by the National Institutes of Health Division of AIDS for participation in clinical trials.[109]

2.1.3 BLT study population

The BLT study was a cross-sectional, clinic-based study conducted at CLI’s McGuire Wellness Center in rural Lilongwe District, Malawi. CLI sees 450 patients in a typical week for genitourinary, respiratory, gastrointestinal and other medical concerns, and for immunizations and family planning services.

Any woman who reported to CLI with any genitourinary symptoms was referred to a study staff member who informed her about the study. If the woman was interested in hearing more, the research assistant administered an eligibility form. A woman was eligible for enrollment in BLT if she spoke Chichewa, consented to be examined and give biological specimens for testing, was 18-49 years of age, resided in Lilongwe District, and was seeking care for genitourinary symptoms. Genitourinary symptoms were broadly defined as abnormal menstrual cycle or patterns of bleeding; pain with urination, pain during sex, abdominal pain, lower back pain, or any type of pelvic pain; incontinence or
unusual urine odor, frequency or color; unusual vaginal discharge in terms of quantity, odor, color or consistency. Women were excluded if they were menstruating or pregnant. Women who were menstruating were told to return to the clinic the following week if they wished to participate in the study. Enrollment took place from January 2015 to August 2015. A total of 234 women were screened and 200 enrolled in the BLT study.

2.1.4 BLT recruitment

Eligible women underwent the informed consent process and provided written informed consent for the study. All consented women received a participant identification number that was used on all study materials. Study staff also completed a locator form so women could be followed-up with results if they did not return for result visit 2. Women who were eligible were then sent to the research clinician for a pelvic exam and collection of biological specimens. Each woman who participated received 1000 kwacha (~$2.00 USD) following physical examination, specimen collection and interview.

2.1.5 BLT sample size

We enrolled a convenience sample of 200 women in the BLT study. The BLT study was a feasibility study, and the sample size for the study was selected as the maximum number supportable by the study budget.

2.1.6 Data collection

We collected both survey and clinical data.
Survey data. The questionnaire (Appendix A) was administered either before or after the clinical exam depending upon the availability of the study clinician and research assistant. A trained research assistant delivered the questionnaire using the Magpi data collection system (Magpi, Washington, DC) via tablet computers. Questionnaire topics included demographics, reproductive and sexual behavior, cervical cancer and screening, and risk factors for exposure to schistosomal parasites, HIV, UTI and infertility.

Clinical data. At the start of the study the research assistant collected 20ml of urine from participants to test for pregnancy and assess presence of schistosoma eggs and UTIs. All women then underwent a pelvic examination by a trained CLI clinician. Using standard procedures for colposcopic examination, the clinician visualized the vagina and cervix for signs of genital schistosomiasis (epithelial disruptions, ulcers and “sandy patch” lesions). Any abnormalities were recorded on the clinical exam form. While the woman was positioned for the pelvic exam, five swabs were collected to detect a) chlamydia and gonorrhea; b) bacterial vaginosis; c) candidiasis; d) trichomoniasis; and e) HPV typing. After the pelvic exam, the study clinician also collected 10ml blood via venipuncture to detect a) HIV (by rapid test) b) syphilis and c) HSV-2. Prior to the pelvic exam, women were given the option to self-collect a vaginal specimen for HPV testing. If a woman consented to self-collect a vaginal sample she received instructions from the clinician and went behind a privacy screen to collect the sample and then handed it back to the clinician. The clinician remained in the room to answer any questions. The results from these tests were recorded on the results form as they came back to the research team from the CLI and UNC Project labs.
During colposcopy, the study clinician also performed visual inspection of the cervix with acetic acid (VIA). During VIA, 3-5% acetic acid was applied to the cervix with a large cotton swab and left for 60 seconds. Precancerous lesions turn white when combined with acetic acid. Normal cervixes without any precancerous lesions do not change color. Women in whom potentially abnormal lesions were detected were referred for care at the closest tertiary referral hospital (Kamuzu Central Hospital or Bwaila Hospital in Lilongwe). Results from VIA were recorded on the clinical exam form.

**Biological specimen testing and treatment:** Only the results of rapid HIV tests were known on the day of the enrollment visit. Samples sent to reference laboratory for testing (at UNC Project in Lilongwe) were stored under recommended conditions and transported for testing as often as needed to insure high specimen quality. Samples tested were as follows:

- **HIV:** via government-provided rapid tests (e.g. Determine or Unigold)
- **Syphilis:** by reactive rapid plasma regain (RPR) and confirmed by Treponema pallidum particle agglutination assay (TPPA) testing
- **HSV-2:** by ELISA
- **HPV:** by Cepheid GeneXpert HPV assay, which specifies three possible outcomes: HPV type 16, HPV 18/45, and a single composite result capturing HPV 31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68.
- **Chlamydia:** by nucleic acid amplification testing
- **Gonorrhea:** by nucleic acid amplification testing
- **Trichomoniasis:** by microscopy or culture
- **Bacterial vaginosis:** by Nugent scoring of gram-stained vaginal smears
- **Candida:** by gram stain
- **Bacterial urinary tract infection:** by dipstick urinalysis and quantitative urine culture
- **Urinary schistosomiasis:** by microscopy
2.2 UTHA cohort study methods

2.2.1 UTHA study setting

The UTHA cohort study took place in the catchment area of the McGuire Wellness Center. This study used two-stage stratified, clustered sampling. Study staff conducted a census of the catchment area in Summer/Autumn 2013 that enumerated all households in the catchment area by village and served as the sampling frame. Villages were stratified by trading centers, plantations and rural villages. The study team then created village clusters (combining some small villages into single clusters) consisting of between 50-250 households. Clusters were randomly selected within each strata until a total of 1,000 households were included.

2.2.2 Study population

The villages range from under 1 kilometer to around 25 kilometers away from the clinic. The education level of the villagers was generally low with many in the catchment area attending only the first few years of primary school or having no schooling at all. Most of the community members were sustenance farmers.

Women living in the randomly sampled villages in the catchment area were eligible to participate in the cohort study. Inclusion criteria included being reproductive age (15-39 years), residing in Lilongwe District, and being able to give informed consent. For all participating women, male partners were eligible to participate in the study if they were at least 15 years of age.
2.2.3 Recruitment

Research assistants went to each household in the selected villages. For women who agreed to participate, their male partners were also invited to participate in the study. Each participant received 1,000 Kwacha (~$2.00 USD) upon the completion of the questionnaire. Among participants, 75% had a monthly income of 29,000 kwacha or lower. We selected the compensation amount in discussion with Malawian clinicians and research assistants and it is comparable to compensation amounts for other studies in nearby areas.

All participants were read aloud an informed consent document and those who consented signed or thumb printed the document prior to participation in the study. Those participants under the age of 18 gave their assent to participate and a guardian consented for 15-17 year olds.

2.2.4 Sample size

The study was powered to detect an association between decision making and HIV testing. We anticipated correlation within villages and within households that reduced our effective sample size, so we based it on an estimate of 500 independent observations, rather than the 1,000 women who will be interviewed. Based on previous research in rural Malawi, we expected that ~70% of our participants would have had an HIV test in the past (DHS 2010).[110] With 500 observations, we would have 80% power (with α=0.05) to detect an odds ratio of 1.23 or greater for having had an HIV test, comparing two groups that differ by one standard deviation in a decision-making
index. To reach approximately 1,000 women of reproductive age we enrolled women from 15 villages, 3 rural trading centers, and 1 plantation. In total 1,475 participants were enrolled: 1,034 women and 441 men.

2.2.5 Data collection

Trained research team members conducted face-to-face interviews with consented participants using tablets. The interview lasted approximately 70 minutes and included questions on decision making processes, healthcare utilization and questions on sexual and reproductive health (Appendix B). Biometric measurements (weight, height, mid-upper arm circumference, blood pressure) were also assessed and recorded. Upon completion of the survey, we asked permission to re-contact participants in 4-8 months.

2.2.6 Data management

Research assistants recorded answers directly onto tablet computers using the Magpi data collection system (Magpi, Washington, DC) and uploaded to an internet-based storage system every evening.
Chapter 3: Intravaginal practices among a cohort of rural Malawian women

3.1 Abstract

Background: Intravaginal practices (IVP) are highly prevalent and commonly performed in many countries for a variety of purposes related to genital health, hygiene, and sexual pleasure. However, IVP may also have harmful side effects, including associations with bacterial vaginosis and HIV.

Methods: We characterized the prevalence and motivations for IVP among 650 women participating in the baseline survey of a community-based cohort study on sexual and reproductive health in rural Lilongwe District, Malawi. Key variables included the type and frequency of IVP, and motivations for engaging in IVP.

Results: Most women (95%) had engaged in IVP in the past 30 days: 88% reported internal vaginal cleansing with water only, 87% reported cleansing with soap and water, and 84% reported inserting cotton, cloth or tissue. A majority (60%) reported at least three practices. Very frequent engagement in at least one type of IVP was also common: among those who inserted cotton, cloth or tissue, 43% did so more than once a day; among those who cleansed internally with soap and water, 51% did so more than once a day. Women reported many reasons for using IVP. The most commonly reported reasons were to remove odors (91%), to
remove extra moisture (58%), to prevent disease (49%), to relieve symptoms of disease (41%), and to improve sex for partner (40%).

Conclusions: IVP are highly prevalent and frequently performed among these rural Malawian women. Future research should investigate the associations between IVP and STI prevalence.

3.2 Introduction

Intravaginal practices (IVP) is a term that describes a wide range of practices that women undertake to modify the vaginal environment. Intravaginal practices can include internally cleansing the vagina with liquids; inserting dry substances to absorb vaginal fluid or “tighten” the vagina; ingesting substances intended to affect the feel or smell of the vagina; and inserting herbs and other substances for various purposes such as improving sexual intercourse or as treatment for vaginal discharge.[40,43,111,112] Women engage in IVP for a variety of purposes related to maintaining genital health, personal hygiene and sexuality.[40–44] Different practices may be used to fulfill different objectives. For example, many women engage in IVP to dry the vagina. In some regions women view a dry vagina as proof of fidelity within partnerships and to please male sexual partners.[47] Some women associate dryness with health, as common vaginal infections are associated with discharge and thus use IVP for hygiene.[44]

Unfortunately, IVP have also been associated with unintended and harmful side effects. Vaginal cleansing and insertion of detergents or other materials into the vagina can disrupt the genital mucosa and perturb the vaginal lactobacilli that populate a healthy vagina, putting women at increased risk for various poor health outcomes.[111,113] Previous research is mixed, but supports modest associations between IVP and bacterial vaginosis (BV), HIV,
preterm delivery, and spontaneous abortion.[48,113,114] More limited evidence also suggests an association between IVP and cervical cancer, ectopic pregnancy, and other vaginal infections like candidiasis. [42,48,111] Both type, depending on causticity of IVP such as household cleaners vs. water, and frequency, with more frequent IVP leading to continual disruptions of the vaginal microbiome, may play a role in development of these outcomes.[48]

Given these health implications, the high prevalence of IPV in many settings is of public health concern. In some African countries, up to 97% of women report some type of IVP.[48] Studies in Zimbabwe, Zambia, Kenya, South Africa, and Tanzania found that a majority of women report at least one type of IVP.[115–119] While IVP are widely used throughout sub-Saharan Africa and the rest of the world, the types of practices, frequency of use, and motivations for IVP are culturally specific and widely varied. Therefore, our objective was to characterize the prevalence and motivations for IVP among women participating in the baseline survey of a community-based cohort study on sexual and reproductive health in rural Lilongwe District, Malawi.

3.3 Methods

3.3.1 Study setting and participants

The baseline wave of the cohort study included 1,034 women and their male sexual partners, recruited from the catchment area of a clinic in rural Lilongwe District, Malawi, between July 2014 to February 2015.[120]. Briefly, we used two-stage stratified cluster sampling to select villages to permit enrollment of a minimum of 1,000 reproductive-age women. All women between the ages of 15 and 39 years who lived in selected villages were
eligible to participate. This analysis focuses on a subset of women (n=650) who answered an ancillary data collection module about IVP.

3.3.2 Data collection

After participants provided informed consent, trained research assistants administered a face-to-face questionnaire. The survey covered a broad range of sexual and reproductive health topics and basic demographic information. Interviewers recorded answers directly onto tablets using the Magpi data collection system (Magpi, Washington, DC) and data were uploaded to an internet-based storage system every evening.

3.3.3 Analysis

Type and frequency. To assess women’s engagement in IVP, we asked participants whether they cleansed, inserted, or applied any of the following into the vagina in the past 30 days: water only; soap and water; cotton, cloth or tissue; alum or other powder; herbs, leaves, or castor oil; or lemon juice. We also asked about application of any of these substances on the labia. All women were informed that, with the exception of the questions specifically about the labia, all other questions were focused on internal cleansing or insertion of substances. For any practice that women reported, we assessed the frequency with which they did the particular practice (more than once a day, about once a day, between once a day and once a week, between once a week and once a month or once a month or less often).

We then characterized separate, unadjusted associations between several demographic characteristics identified from the literature and overall IVP frequency. Given the high
frequency of reported IVP, we collapsed women’s responses into three frequency categories: those who engaged in any IVP a) at least once a day or more often, b) less than once a day, or c) never. Because internal cleansing with water only has been shown repeatedly not to increase risk of adverse outcomes [121–123], women who reported only this behavior were classified in the ‘never’ frequency group. Many women reported multiple types of IVP, so the overall IVP frequency variable captures the most frequent practice reported by each woman. For example, if she cleansed with soap and water more than once a day, but only inserted cotton, cloth or tissue once a week, she was classified in the “more than once a day” group.

We assessed participant characteristics including age, education, self-reported HIV status, relationship status (married/partnered vs. single), current hormonal contraception use, self-report of any STIs (lifetime), self-report of abnormal vaginal discharge in the past 12 months, self-report of genital ulcers in the past 12 months, parity, number of sexual partners, time since last intercourse, and sexual frequency in the past month.[43,114,116,117] We used chi-square statistics to evaluate differences in participant characteristics between frequent users (at least once a day), less frequent users (less than once a day) and non-users of IVP.

Motivations for IVP. Last, we calculated frequencies of reported motivations for IVP. Women who reported at least one IVP were asked about their motivations for IVP using a prompted list. Options included: to get ready for sex, to clean after sex, to avoid pregnancy, to improve sex for partner, to improve sex for self, to please sexual partner, to tighten the vagina, to remove odors, to remove extra moisture or fluid, to prevent disease, and to relieve symptoms of disease. All data were analyzed using Stata 12.0 (StataCorp, College Station, Texas).
Due to a skip pattern error during data collection, only 139 of 345 women in four of the 20 sampled villages answered the IVP module in the survey. Because the exclusion of these women was driven by their responses to an unrelated, earlier question in the survey and was therefore non-random, we excluded all women from these four villages from the analysis dataset. The women included in the analysis were similar to excluded women except that they had lower education, higher reported condom use and lower reported history of genital ulcers than the included women. Among the women in the excluded villages who answered the IVP module, the prevalence and frequency of IVP did not differ significantly from the women from villages where all participants answered the IVP module.

3.3.4 Ethical approval

This project received ethical approval from the Ohio State University Institutional Review Board and the University of Malawi College of Medicine Research and Ethics Committee.

3.4 Results

Of 650 women included in this analysis, most were married (81%) and generally had low levels of education, with a median of 5 years of schooling. Participants’ median age was 25 years (Table 1). Ten percent of women self-reported ever having an STI, while 12% reported abnormal genital discharge in the preceding 12 months and 6% reported genital ulcers in the past 12 months.
3.4.1 Types of intravaginal practices

IVP in the past 30 days was extremely common: 88% reported internal cleansing with water only, 87% reported cleansing with soap and water, and 84% reported inserting cotton, cloth or tissue to cleanse the vagina. Less commonly reported practices included the application of substances on the external genitalia (10%), application of castor oil (2%), the internal insertion of alum or powder (2%), and the insertion of herbs (1%). Only 5% of women reported no IVP, and most (60%) reported three or more different practices (including use of water only).

3.4.2 Frequency of IVP

Approximately half of participants reported very frequent engagement in at least one type of intravaginal practice: 43% reported inserting cotton, cloth or tissue more than once a day; 51% reported internal cleansing with soap and water more than once a day; and 54% reported cleansing with only water more than once a day (Table 2). Among those women (n=82) who reported any other IVP (including application of substances on external genitalia, application of castor oil and insertion of alum, powder or herbs), 48% reported using these practices at least once a day.

The characteristics of women who performed IVP at least once a day, less often than once a day and never (or water only) were similar overall, with significant differences only in time since last intercourse, self-reported HIV status, abnormal genital discharge in the last 12 months, and age (Table 1). Seventy-nine percent of the highest frequency group and 77% of the moderate frequency group reported having intercourse within the past week, while only 53% of those never engaging in IVP reported intercourse within the past week (p<0.001).
Women in the ‘never’ group also had a higher self-reported prevalence of HIV (1% for high frequency vs. 2% for moderate frequency vs. 14% for never users, p<0.001) and abnormal genital discharge in the last 12 months (11% for high frequency vs. 16% for moderate frequency vs. 33% for never users, p=0.001).

3.4.3 Motivations for intravaginal practices

Participants cited many reasons for performing intravaginal practices (Table 3). The five most-commonly reported reasons were to remove odors (91%), to remove extra moisture (58%), to prevent disease (49%), to relieve symptoms of disease (41%), and to improve sex for partner (40%). The median number of reasons given for performing IVP was 3 (range 0-11).

3.5 Discussion

Intravaginal practices are potentially associated with a host of harmful health outcomes. With an HIV prevalence of 10% in Malawi [124], it is important to gain a better understanding of this potentially modifiable risk factor for HIV susceptibility and transmission. In this sample of rural Malawian women we found that IVP are highly prevalent and frequently performed. Perhaps because of the generally high prevalence and frequency of IVP across the study sample, it was not possible to identify demographic characteristics other than younger age that differed between women with high and low IVP frequency. Only lower prevalence of self-reported HIV status, lower prevalence of self-reported abnormal genital discharge in the last 12 months, and less time since last intercourse significantly varied
between women reporting high vs. low IVP frequency. We also found that motivations for IVP varied, with many women reporting multiple reasons that related to genital health, cleansing, and pleasing their sexual partner.

Our findings of high frequency of IVP are in line with research in other settings. One study among HIV-infected Zambian women found that 93% of participants reported engaging in some type of practice.[115] In HIV-uninfected Zimbabwean women, 84% of participants reported engaging in IVP.[117] Across all settings in a multi-country study in Mozambique, South Africa, Indonesia, and Thailand, more than 60% of women reported some sort of IVP.[43] Similar to our findings, other studies have reported that IVP prevalence varies according to the type of practice. In an IVP cessation study among HIV-uninfected Kenyan women, prevalence of practices ranged from a high of 59% for cleansing with soapy water to 7% of women inserting alum powder, brewed tea, or other substances like cream or herbs.[125] This high frequency with which IVP are performed may be important for risk of BV, STI, and HIV by causing continual disruption of the vaginal microflora, cervical mucosa, or vaginal pH.[126]

Associations with IVPs in our study were mostly in line with previous research finding few factors associated with IVP. Among female sex workers in Kenya only higher education, younger age, and being divorced or widowed were associated with IVP.[116] In contrast to our study, among these women, time since last intercourse was not associated with IVP, perhaps because of the different populations (sex workers vs. community based women). Other research in HIV-infected women in Zambia found that ever use of IVP was associated with an increased odds of abnormal vaginal discharge.[115] Interestingly, we found that less
frequent IVP was associated with reporting abnormal vaginal discharge, illustrating that in this population, IVP appear to work, or at least may appear to do so to users of IVP. This finding could also alternatively illustrate how women get BV and vaginal discharge (perhaps as a result of IVP), and are then in a cycle of doing IVP to control the discharge thinking the IVP is preventing the discharge, rather than recognizing it may be the cause. Future, longitudinal research is necessary to better understand this association.

Our study found that IVP were used for various reasons and most women used them to remove odors. Other studies also found that women report multiple reasons for using IVP and motivations were associated with the type of practice. In a qualitative study among Zimbabwean and Malawian women, participants reported multiple reasons for using IVP, including to please a sexual partner and to keep the vagina clean and healthy.[47] Among female sex workers in Kenya, IVP was performed for hygiene (100%), to tighten the vagina (41%), and to prevent infection (40%).[116] In the multi-country study, Mozambican women reported a variety of reasons for various types of IVP, including to enhance male sexual pleasure (57%) and to treat infections or symptoms (44%).[43] The women reported different reasons for different practices, with women ingesting certain substances to increase male sexual pleasure and inserting traditional herbs to treat infection.[43] Zambian women also reported multiple reasons motivating IVP, with 90% citing hygiene, 72% giving health as a motivation, and 35% describing partner pleasure as a reason for IVP.[115] The majority of women in that study reported that cleansing with water (100%), soap (100%), and fabric or cotton (92%) was for hygiene-related reasons, while traditional medicines (67%) and herbs (68%) were used for partner pleasure.[115]
As with many studies on IVP, we relied on self-reported behavioral data, which can result in social desirability and recall bias. However, given the high proportion of women reporting IVP, bias due to self-report appears unlikely to bias our findings. Additionally, we attempted to minimize recall bias by referencing only the past 30 days. We were unable to assess whether particular motivations corresponded to particular practices, because we asked about motivations for all practices rather than after each individual type.

IVP may represent a modifiable risk factor that, if reduced or eliminated, could lead to reduced morbidities relating to BV, HIV and other adverse health outcomes. Given the high prevalence of women engaging in IVP, the population attributable risk of IVP could be significantly reduced if fewer women performed IVP. Previous research has shown that cessation programs can be successful.[125,127,128] However, the prevalent nature of IVP in this rural Malawian population might make cessation programs unsuccessful without an understanding of the motivations for undertaking such practices. Understanding the objective of particularly harmful IVPs can allow for the implementation of safer alternatives. Future research should expand our knowledge on IVP in this population by investigating the association between IVP and STI prevalence, to better quantify the health risks associated with these IVP and inform future interventions for behavior change.
<table>
<thead>
<tr>
<th>Frequency of IVP</th>
<th>Total (n=650)</th>
<th>≥ 1 per day (n=556)</th>
<th>&lt; 1 per day (n=70)</th>
<th>Never (n=24)</th>
<th>p-value$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, med (IQR)</td>
<td>25 (20,31)</td>
<td>24 (20,30)</td>
<td>28 (21,33)</td>
<td>26 (21,31)</td>
<td>0.051</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.589</td>
</tr>
<tr>
<td>None</td>
<td>53 (8)</td>
<td>47 (8)</td>
<td>3 (4)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>Standard 1-8</td>
<td>524 (81)</td>
<td>445 (80)</td>
<td>61 (87)</td>
<td>18 (75)</td>
<td></td>
</tr>
<tr>
<td>Form 1-4</td>
<td>71 (11)</td>
<td>62 (11)</td>
<td>6 (9)</td>
<td>3 (13)</td>
<td></td>
</tr>
<tr>
<td>Self-reported HIV status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Yes</td>
<td>9 (2)</td>
<td>5 (1)</td>
<td>1 (2)</td>
<td>3 (14)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>535 (98)</td>
<td>457 (98)</td>
<td>60 (98)</td>
<td>18 (86)</td>
<td></td>
</tr>
<tr>
<td>Relationship status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.192</td>
</tr>
<tr>
<td>Married/engaged</td>
<td>525 (81)</td>
<td>449 (81)</td>
<td>59 (84)</td>
<td>17 (71)</td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>90 (14)</td>
<td>76 (14)</td>
<td>9 (13)</td>
<td>5 (21)</td>
<td></td>
</tr>
<tr>
<td>Divorced/widowed</td>
<td>35 (5)</td>
<td>31 (6)</td>
<td>2 (3)</td>
<td>2 (8)</td>
<td></td>
</tr>
<tr>
<td>Hormonal contraception (ever)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.274</td>
</tr>
<tr>
<td>Yes</td>
<td>469 (72)</td>
<td>399 (79)</td>
<td>55 (79)</td>
<td>15 (63)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>181 (28)</td>
<td>157 (21)</td>
<td>15 (21)</td>
<td>9 (38)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Participant characteristics from the 650 sampled women in Malawi

continued
Table 1 continued

<table>
<thead>
<tr>
<th>Condom use (ever)</th>
<th>0.239</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>44 (7)</td>
</tr>
<tr>
<td>No</td>
<td>605 (93)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Self-reported past STI (lifetime)</th>
<th>0.178</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>63 (10)</td>
</tr>
<tr>
<td>No</td>
<td>584 (90)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Self-reported abnormal genital discharge (last 12 months)</th>
<th>0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>80 (12)</td>
</tr>
<tr>
<td>No</td>
<td>569 (88)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Self-reported history of genital ulcers (last 12 months)</th>
<th>0.632</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>40 (6)</td>
</tr>
<tr>
<td>No</td>
<td>610 (94)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time since last intercourse</th>
<th>&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within 1 week</td>
<td>425 (78)</td>
</tr>
<tr>
<td>&gt; 1 week and ≤ 1 month</td>
<td>54 (10)</td>
</tr>
<tr>
<td>&gt; 1 month and ≤ 1 year</td>
<td>543 (8)</td>
</tr>
<tr>
<td>&gt; 1 year</td>
<td>11 (2)</td>
</tr>
</tbody>
</table>

continued
Table 1 continued

<table>
<thead>
<tr>
<th>Sexual frequency (past 30 days)</th>
<th>≤ 1 time per week</th>
<th>2-6 times per week</th>
<th>≥ 7 times per week</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>83 (17)</td>
<td>66 (16)</td>
<td>14 (26)</td>
<td>3 (20)</td>
</tr>
<tr>
<td></td>
<td>318 (66)</td>
<td>275 (66)</td>
<td>33 (61)</td>
<td>10 (67)</td>
</tr>
<tr>
<td></td>
<td>83 (17)</td>
<td>74 (18)</td>
<td>7 (13)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Lifetime number of sexual partners, med (IQR)</td>
<td>2 (1,2)</td>
<td>2 (1,2)</td>
<td>2 (1,2)</td>
<td>2 (1,2)</td>
</tr>
<tr>
<td>Number of children, med (IQR)</td>
<td>3 (2,4)</td>
<td>3 (2,4)</td>
<td>3 (2,4)</td>
<td>2.5 (2,3.5)</td>
</tr>
</tbody>
</table>

1 Some categories do not total to 650 due to missing responses.

2 Self-reported; refers to lifetime history of STI

3 \(P\)-values compare the different frequency categories
Table 2: IVP frequency, by individual practice of IVP among 650 women in Malawi.

<table>
<thead>
<tr>
<th>Practice</th>
<th>≥ 1/day n (%)</th>
<th>About once a day n (%)</th>
<th>1/day to 1/week n(%)</th>
<th>1/week to 1/month n(%)</th>
<th>≤ 1/month n(%)</th>
<th>Never n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water only</td>
<td>360 (54)</td>
<td>141 (21)</td>
<td>37 (6)</td>
<td>31 (5)</td>
<td>3 (&lt;1)</td>
<td>78 (12)</td>
</tr>
<tr>
<td>Soap and water</td>
<td>335 (51)</td>
<td>154 (23)</td>
<td>41 (6)</td>
<td>25 (4)</td>
<td>6 (&lt;1)</td>
<td>89 (13)</td>
</tr>
<tr>
<td>Cotton, cloth or tissue</td>
<td>287 (43)</td>
<td>150 (23)</td>
<td>52 (8)</td>
<td>46 (7)</td>
<td>10 (2)</td>
<td>104 (16)</td>
</tr>
<tr>
<td>Other substances²</td>
<td>40 (6)</td>
<td>22 (3)</td>
<td>15 (2)</td>
<td>3 (&lt;1)</td>
<td>2 (&lt;1)</td>
<td>595 (88)</td>
</tr>
</tbody>
</table>

¹Women were allowed to report multiple practices
²Other substances included inserting alum or powder, herbs, castor oil or application of substances on labia
Table 3: Participants motivations for IVP among 650 sampled women in Malawi$^{1,2}$

<table>
<thead>
<tr>
<th>Reason</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>To remove odors</td>
<td>602</td>
<td>91</td>
</tr>
<tr>
<td>To remove extra moisture</td>
<td>386</td>
<td>58</td>
</tr>
<tr>
<td>To prevent disease</td>
<td>327</td>
<td>49</td>
</tr>
<tr>
<td>To relieve symptoms of disease</td>
<td>273</td>
<td>41</td>
</tr>
<tr>
<td>To improve sex for partner</td>
<td>264</td>
<td>40</td>
</tr>
<tr>
<td>To please sexual partner</td>
<td>262</td>
<td>39</td>
</tr>
<tr>
<td>To improve sex for self</td>
<td>258</td>
<td>39</td>
</tr>
<tr>
<td>To clean after sex</td>
<td>251</td>
<td>37</td>
</tr>
<tr>
<td>To tighten the vagina</td>
<td>236</td>
<td>35</td>
</tr>
<tr>
<td>To get ready for sex</td>
<td>194</td>
<td>29</td>
</tr>
<tr>
<td>To avoid pregnancy</td>
<td>61</td>
<td>9</td>
</tr>
</tbody>
</table>

$^1$Women were allowed to provide multiple reasons

$^2$Motivations referred to all IVP in general
Chapter 4: Do intravaginal intravaginal practices increase women’s risk of human papillomavirus?

4.1 Abstract

Background: Many women engage in intravaginal practices (IVP) with a goal of improving genital hygiene and sexual pleasure. However, IVP can disrupt the genital mucosa, possibly increasing acquisition risk of HIV and the reproductive tract infection bacterial vaginosis. Limited prior research also suggests an association between IVP and HPV. In this analysis, we examine associations between IVP and high risk HPV (hr-HPV).

Methods: We included 193 women from a clinic-based study examining sexual and reproductive tract infections in rural Malawi. Using a standardized survey instrument, research assistants asked a series of questions on the type and frequency of IVP, and a study clinician collected an endocervical sample for HPV testing. We calculated prevalence ratios for the association between type, frequency, and combined type and frequency of IVP and hr-HPV.

Results: We found that intravaginal practices were commonly performed with 96% of women reporting use of at least once practice. Among our sample of 193 women, 22%
had any hr-HPV. We did not find a significant association with type of IVP, frequency of IVP, or the combined measure of type and frequency of IVP and hr-HPV.

Conclusions: Our findings suggest that IVP may not be a risk factor for hr-HPV infection. However, the high prevalence and frequency of IVP in our population may have limited our ability to detect significant differences.

4.2 Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection among all women worldwide. Most women will be infected with at least one type of HPV at some point during their lifetime.[1,2] HPV is a DNA virus and includes over 150 different genotypes. It is spread through skin or mucosal contact primarily during vaginal, oral and anal sex.[1,3] There are approximately 40 types of HPV responsible for genital tract infections and these can be classified as “low-risk” or “high-risk” HPV.[3] Low-risk (lr) HPV infections can sometimes cause warts, but are not cancerous. HPV 6 and 11 are the most prevalent lr-HPV types, causing 90% of cases of genital warts.[3] High-risk (hr) HPV is a causative agent for cervical cancer and is also associated with oropharyngeal, vulvar, vaginal, anal, and penile cancer.[3,4] Some types are more oncogenic, such as HPV 16 and 18 which are associated with 70% of all cervical cancer cases worldwide.[6–8] While many women are infected with human papillomavirus (HPV), the majority will clear the infection without any signs, symptoms or lasting impact. There are many factors, such as smoking and co-infections with HIV, that are associated with increasing a woman’s risk of initial infection and progression to persistent hr-HPV infection, cervical
intraepithelial neoplasia (CIN) or cervical cancer.[129,130] A limited literature suggests intravaginal practices (IVP) may be associated with increased prevalence of HPV and it is possible that these practices could also affect progression to cervical cancer.

IVP encompass a wide range of practices that women may undertake for a variety of purposes including maintaining genital health, personal hygiene, and enhancing sexual experience.[40,43,47,48] Types of IVP vary across and within countries. For example, women more commonly report cleansing with commercial products in Thailand and cleansing with household products like soap or aspirin in South Africa.[40] Women who perform IVP may use multiple substances simultaneously or at different times[43] and the frequency of IVP may vary by type and purpose of the practice. Some women use certain practices, like vaginal douching, only once a month.[48] The same women may use other practices, like inserting cloth, multiple times daily. In Malawi, IVP are common with 95% of women reporting at least one practice in the previous 30 days.[131]

Our objective was to assess the associations between type and frequency of IVP, and detection of hr-HPV and precancerous lesions in a sample of care-seeking women with a high prevalence of IVP in rural Lilongwe District, Malawi.

4.3 Methods

4.3.1 Study design and setting

We enrolled women into a cross-sectional, clinic-based study on sexual and reproductive tract infections conducted from January to August 2015. Non-pregnant
women between 18 and 49 years of age seeking care for genitourinary symptoms at a
clinic in rural Lilongwe District, Malawi were eligible to participate.

4.3.2 Data collection

Each participant underwent a pelvic exam during which the study clinician
collected a cervical swab for HPV testing. Cervical swabs were tested using the
GeneXpert HPV assay (Cepheid, Sunnyvale, CA). During the exam the clinician also
performed cervical cancer screening using visual inspection of the cervix with acetic acid
(VIA). With VIA, potential lesions are identified by acetowhite areas on the cervix. As
part of the main study we also screened for HSV-2, HIV (Uni-Gold, Trinity Biotech,
Jamestown, NY), and gonorrhea (ProbeTec, BD Diagnostics, Sparks, MD). Following the
exam, a trained research assistant conducted a brief questionnaire via tablet computer to
assess the timing (before sex, after sex, both times, neither), type, and frequency of IVP
and other sexual and reproductive factors. Survey data were collected using the Magpi
data collection system (Magpi, Washington, DC).

4.3.3 Measures

Outcomes. Our primary outcome was prevalence of hr-HPV. There were four
possible outcomes for HPV testing: (a) negative for hr-HPV; (b) positive for hr-HPV type
16, (c) positive for hr-HPV types 18/45; or (d) positive for 11 other pooled hr-HPV types
(31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68). For this analysis, because of our limited
sample size, we combined all types of hr-HPV into a binary variable (positive/negative).
As a secondary outcome we examined the prevalence of hr-HPV and abnormal cervical lesions (positive/negative) detected through VIA with four possible outcomes: (1) VIA positive, hr-HPV positive; (2) VIA negative, hr-HPV positive; (3) VIA positive, hr-HPV negative; and (4) VIA negative, hr-HPV negative.

Exposures. Our primary exposures included participant self-report of engagement in different types of IVP (cleansing with soap and water; cleansing with cotton, cloth or tissue; inserting alum or other powder, herbs, leaves, castor oil, or any other vaginal products from a traditional healer or herbalist) and frequency of each practice (more than once a day, once a day, a few times per week, a few times per month, once a month or less often, never). Based on the distribution of the data, for the primary analysis we categorized frequency into three categories: more than once per day, once per day or less often, and never. When examining IVP frequency overall, if women reported multiple types of IVP they were categorized according to their most frequent practice.

4.3.4 Analysis.

All clinical and laboratory data were first captured on paper forms and were subsequently entered into an internet-based data storage system. Laboratory and survey data were cleaned and analyzed using Stata 14 (StataCorp, College Station, TX) and R 3.2.2 (R Core Team, Vienna, Austria). After first describing the prevalence of IVP and hr-HPV in the study population, we then examined associations between IVP type and frequency of IVP, and hr-HPV
Type of IVP. As our primary outcome (positive hr-HPV) was found in greater than 10% of the study population, we calculated prevalence ratios (PRs) rather than odds ratios.[132] We used a generalized linear model with a binomial distribution and log link to examine the association between type of practice (cleansing with soap and water, cleansing with cotton, cloth or tissue, and inserting any other products) and prevalent hr-HPV. We only assessed unadjusted associations as adjustment for other variables led to imprecise estimates due to small cell sizes.

Frequency of IVP. To assess the association between frequency of IVP and hr-HPV we ran three separate unadjusted models, one for each IVP type (cleansing with soap and water, cleansing with cotton, cloth, or tissue, and any IVP). Due to the small number of participants who reported using any substance other than soap, cotton, cloth and tissue, we were not able to include this other group in a separate regression analysis. We created a directed acyclic graph [133] based on existing literature to identify potential confounders of the association between IVP and hr-HPV. We retained variables as confounders in the fully-adjusted multivariable model based on a change-in-estimate criterion: if removal of the variable led to a change of greater than 10% in the association between the main independent variable and the outcome, it was retained in the model. We tested for age, education, household income, lifetime number of sexual partners, age at first sex, and STI diagnosis. Our final models adjusted for number of lifetime sexual partners and age at first sex. Any variable found to confound the association of interest in any model was also retained in the other multivariable models, to ease interpretability of adjusted estimates.
Type and frequency of IVP. In order to examine the joint effects of IVP type and IVP frequency, we created a new categorical variable combining these measures. A woman was classified into one of five categories: 1) high frequency (>1/day) for both cleansing with soap and water and with cotton, cloth or tissue; 2) high frequency for cleansing with soap and water and low frequency (≤1/day) for cleansing with cotton, cloth, or tissue; 3) low frequency for soap and water and high frequency for cotton, cloth or tissue; 4) low frequency for both practices; 5) reports neither. As we had small cell sizes for some IVP categories, we limited our analysis to unadjusted models only. Finally, we used Fisher’s exact test [133] to assess the association between IVP type and frequency and the presence of hr-HPV and VIA results, allowing for four potential outcomes: hr-HPV positive and VIA positive; hr-HPV positive and VIA negative; hr-HPV negative and VIA positive; hr-HPV negative and VIA negative.

4.3.5 Ethical approval

This project received ethical approval from the Ohio State University Institutional Review Board and the University of Malawi College of Medicine Research and Ethics Committee.

4.4 Results

4.4.1 Participant characteristics

We screened a total of 234 women and enrolled 200 women in the study. We included 193 women in this analysis (97% of overall sample). Of the seven women who
were not included, one was enrolled before HPV testing supplies arrived at the clinic and 6 women’s samples were not tested due to a shortage of testing kits. Among the 193 women included in the analysis, the median age of participants was 33 years (interquartile range (IQR): 29, 38) and 34% of women had at least some secondary education (Table 4). Nearly all women were married (93%) and they reported a median of 2 lifetime sexual partners (IQR 1, 3).

Three percent of women (n=6) were infected with HIV, and 5% tested positive for gonorrhea at the time of their visit. Among women included in our study, 51% were HSV-2 seropositive.

Twenty-two percent (n=42) of participants tested positive for hr-HPV infection, some of whom had multiple categories of hr-HPV types (Table 4). Nine percent of women (n=16) had an abnormal lesion identified during VIA.

IVP were very common in the sample, reported by 96% of women (Table 5). Cleansing with cotton, cloth, or tissue was most commonly reported (94%) followed by cleansing with soap and water (47%). Very few women (5%) reported using substances other than soap, cotton, cloth, or tissue. Among women reporting any IVP, these practices were frequent: 61% of women who cleansed with soap and water did so more than once per day, and 67% of women who cleansed with cotton, cloth or tissue did so more than once per day.
4.4.2 Associations between IVP and hr-HPV

We found no statistically significant association between IVP type and hr-HPV (cleansing with soap and water PR: 1.03, 95% CI: 0.51, 2.08; cleansing with cotton, cloth or tissue PR 0.73, 95% CI: 0.25, 2.08; other products PR: 1.96, 95% CI: 0.69, 5.5; Figure 5). We also found no association between IVP frequency and hr-HPV in unadjusted or adjusted models focusing on any IVP (PR for ≤1/day: 0.96, 95% CI:(0.09, 3.66); PR for never: 0.57, 95% CI: (0.51, 1.82)) or separately for cleansing with soap and water, or cleansing with cotton, cloth or tissue (Table 6).

Examining IVP type and frequency combined, we did not find any significant associations between the combined exposure variable and hr-HPV (low frequency PR 0.66, 95% CI: 0.32, 1.35; Table 7). However, all categories had a PR below 1 when compared to high frequency for both types of IVP. We also found no significant association between IVP frequency and the combined hr-HPV and VIA (p =0.43).

4.5 Discussion

IVP are associated with bacterial vaginosis and potentially other STI transmission, including HPV. Examining these potential associations is important in a population with a high prevalence and frequency of IVP, such as our study population. While there are limited data on the prevalence of HPV among Malawian women, our prevalence estimate is in line with other research.[13,15] In our study, we found no significant association between IVP and hr-HPV when examining the association by both type of IVP and frequency of IVP, nor did we find an association between IVP and
abnormal cervical lesions. In an analysis using a composite variable capturing all IVP, we observed a non-significant trend of decreasing odds of hr-HPV as frequency of IVP decreased. However, when practices were examined separately we did not see the same pattern, nor did we see significant associations when comparing different risk profiles of IVP users.

Our findings are similar to some previous studies on this topic. A cross-sectional study of female sex workers in Burkina Faso found no significant association between douching and HPV (including both low-risk and high-risk types).[52] In a study of low-risk women in the United States, there was no significant association identified when examining different douching preparations (chemical, commercial, water/vinegar, water/soda, water only) and cervical carcinoma.[56]

Other studies have found associations between high frequency of IVP and HPV or abnormal lesions. However, the significance and the strength of the association varies depending on what is defined as high frequency and the low-frequency or unexposed comparison group.[42,48,56,57] A meta-analysis found a weak overall association between douching and abnormal cytology, and among women who douchered more frequently (at least once a week), the pooled adjusted relative risk was 1.86 (95% CI: 1.29, 2.68).[42] However, similar to our findings, that report did not find a significant association between those who douched less frequently compared to those who never douched.[56] As the majority of women in our study reported a high frequency of IVP, we may not have had a sufficient number of women reporting a low frequency of IVP to detect significant differences.
One of the challenges in assessing the risks associated with IVP is in measurement of IVP as they can vary by type and frequency. IVP are very culturally specific and some practices may only be used in different regions. The type and frequency of IVP are most likely both important for risk of disease development. Some studies focus only on comparing different types of practices. Among studies examining type of IVP, douching with commercial products, lemon, or lime juice were all significantly associated with abnormal cytology or cervical cancer.[41,42,52,53,57] Other findings did not show an increased risk of cervical cancer with only use of water or vinegar.[42] Our null findings examining type of practice and hr-HPV may be due in part to the differences in substances used. In the studies identifying significant associations, women used commercial preparations or lemon or lime juice, which may be more abrasive than soap and water or cotton, cloth, or tissue that were reported by women included in our study. We did not have enough women reporting inserting other substances to examine the association with hr-HPV using a potentially more caustic substance.

In contrast to previous studies, we created risk-profiles to incorporate both type and frequency of IVP to obtain a better estimate of the association with hr-HPV and abnormal cervical lesions. While we did not find any statistically significant associations, all estimates showed reduced prevalence of hr-HPV compared to those women reporting high frequency of both practices. This finding suggests that the combination of type and high frequency may play a role in increased hr-HPV infections.

Our study is one of the first to examine the association between IVP and hr-HPV and extends previous research by examining the effect of both the type and frequency of
IVP. However, our findings should be interpreted in light of study limitations. As a pilot study, our sample size included 193 women, which limited our power to assess key associations of interest. Nearly half of women reported multiple IVP; thus, we were unable to fully separate the effects of different practices, as we did not have a sufficient number of women who reported no practices or only one practice. Additionally, due to the high frequency of IVP, we collapsed the different frequencies of IVP, which may have introduced differential misclassification bias. As we combined women who reported IVP about once a day with the lower frequency category we may have underestimated the true association as their risk of hr-HPV may be more similar to those who perform IVP more than once per day. Our sample also recruited women reporting to a clinic with genitourinary symptoms, and as IVP are sometimes undertaken to treat symptoms of STI, it is possible that the high prevalence of IVP may not be generalizable to the rest of the population. However, previous research similarly showed high prevalence of IVP amongst a non-clinic sample in this region.\[43,115,117\]

In this study, among a sample of care-seeking, rural Malawian women we found frequent engagement in a variety of IVP. Our findings on the prevalence of hr-HPV and precancerous lesions were in line with other research in similar settings. However, we did not find significant associations between IVP and hr-HPV, suggesting that the two intravaginal practices we examined may not be a risk factor for HPV infection. Future research examining a larger population to assess the impact of potentially more harmful IVP should be conducted to gain a better understanding of these associations.
Table 4: Participant characteristics of 193 care-seeking women in rural Malawi

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (med, IQR)</td>
<td>33</td>
<td>(29, 38)</td>
</tr>
<tr>
<td>Married</td>
<td>179</td>
<td>(93)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 years</td>
<td>20</td>
<td>(10)</td>
</tr>
<tr>
<td>2-4 years</td>
<td>30</td>
<td>(15)</td>
</tr>
<tr>
<td>5-8 years</td>
<td>78</td>
<td>(40)</td>
</tr>
<tr>
<td>Some secondary schooling</td>
<td>65</td>
<td>(34)</td>
</tr>
<tr>
<td>Number of lifetime sexual partners (med, IQR)</td>
<td>2</td>
<td>(1, 3)</td>
</tr>
<tr>
<td>Age at first sex (med, IQR)</td>
<td>18</td>
<td>(16, 19)</td>
</tr>
<tr>
<td>HPV&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hr-HPV positive</td>
<td>42</td>
<td>(22)</td>
</tr>
<tr>
<td>HPV 16-positive</td>
<td>8</td>
<td>(4)</td>
</tr>
<tr>
<td>HPV 18/45-positive</td>
<td>10</td>
<td>(5)</td>
</tr>
<tr>
<td>Other hr-HPV-positive</td>
<td>29</td>
<td>(15)</td>
</tr>
<tr>
<td>Abnormal cervical lesions</td>
<td>16</td>
<td>(9)</td>
</tr>
<tr>
<td>HIV-seropositive</td>
<td>6</td>
<td>(3)</td>
</tr>
<tr>
<td>HSV-2-seropositive</td>
<td>92</td>
<td>(51)</td>
</tr>
<tr>
<td>Gonorrhea</td>
<td>9</td>
<td>(5)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Results from GeneXpert HPV assay

IQR=interquartile range; HPV=human papillomavirus; HIV=human immunodeficiency virus; HSV=herpes simplex virus
<table>
<thead>
<tr>
<th>Practice</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No IVP</td>
<td>8</td>
<td>(4)</td>
</tr>
<tr>
<td>Cleansing with water</td>
<td>188</td>
<td>(95)</td>
</tr>
<tr>
<td>Cleansing with soap and water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than once per day</td>
<td>57</td>
<td>(29)</td>
</tr>
<tr>
<td>Once per day or less often</td>
<td>37</td>
<td>(19)</td>
</tr>
<tr>
<td>Never</td>
<td>104</td>
<td>(52)</td>
</tr>
<tr>
<td>Timing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before sex</td>
<td>32</td>
<td>(34)</td>
</tr>
<tr>
<td>After sex</td>
<td>21</td>
<td>(22)</td>
</tr>
<tr>
<td>Both times</td>
<td>36</td>
<td>(38)</td>
</tr>
<tr>
<td>Neither time</td>
<td>6</td>
<td>(6)</td>
</tr>
<tr>
<td>Cleansing with cotton, cloth or tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than once per day</td>
<td>125</td>
<td>(63)</td>
</tr>
<tr>
<td>Once per day or less often</td>
<td>61</td>
<td>(31)</td>
</tr>
<tr>
<td>Never</td>
<td>12</td>
<td>(6)</td>
</tr>
<tr>
<td>Timing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before sex</td>
<td>15</td>
<td>(8)</td>
</tr>
<tr>
<td>After sex</td>
<td>72</td>
<td>(38)</td>
</tr>
<tr>
<td>Both times</td>
<td>95</td>
<td>(51)</td>
</tr>
<tr>
<td>Neither time</td>
<td>5</td>
<td>(3)</td>
</tr>
<tr>
<td>Inserting other products</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than once per day</td>
<td>1</td>
<td>(&lt;1)</td>
</tr>
<tr>
<td>Once per day or less often</td>
<td>9</td>
<td>(5)</td>
</tr>
<tr>
<td>Never</td>
<td>188</td>
<td>(95)</td>
</tr>
<tr>
<td>Timing</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before sex</td>
<td>6</td>
<td>(55)</td>
</tr>
<tr>
<td>After sex</td>
<td>3</td>
<td>(27)</td>
</tr>
<tr>
<td>Both times</td>
<td>2</td>
<td>(18)</td>
</tr>
<tr>
<td>Neither time</td>
<td>0</td>
<td>(0)</td>
</tr>
</tbody>
</table>

IVP=intravaginal practices

Timing is only among those who reported doing that practice
Figure 5: Unadjusted prevalence ratios comparing different types of IVP and hr-HPV

1 Prevalence ratios > 1.0 indicates increased risk of hr-HPV with a particular type of IVP vs. not using that type
Table 6: Associations between frequency of IVP and hr-HPV

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted PR (95% CI)</th>
<th>Adjusted&lt;sup&gt;a&lt;/sup&gt; PR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Any IVP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1/day</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>≤ 1/day</td>
<td>0.96 (0.09, 3.66)</td>
<td>0.87 (0.45, 1.68)</td>
</tr>
<tr>
<td>Never</td>
<td>0.57 (0.51, 1.82)</td>
<td>0.84 (0.13, 5.28)</td>
</tr>
<tr>
<td>Soap and water</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1/day</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>≤ 1/day</td>
<td>0.46 (0.18, 1.15)</td>
<td>0.51 (0.20, 1.26)</td>
</tr>
<tr>
<td>Never</td>
<td>0.69 (0.39, 1.23)</td>
<td>0.80 (0.44, 1.45)</td>
</tr>
<tr>
<td>Cotton, cloth or</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1/day</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>≤ 1/day</td>
<td>0.95 (0.51, 1.74)</td>
<td>0.86 (0.46, 1.61)</td>
</tr>
<tr>
<td>Never</td>
<td>1.27 (0.45, 3.54)</td>
<td>1.54 (0.56, 4.26)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Adjusted for lifetime number of sexual partners and age at first sex

IVP=intravaginal practices; hr-HPV=high-risk human papillomavirus; PR=prevalence ratio; CI=confidence interval

Table 7: Unadjusted associations between IVP profile and hr-HPV

<table>
<thead>
<tr>
<th></th>
<th>PR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High&lt;sup&gt;a&lt;/sup&gt; frequency soap/High frequency cotton, cloth, tissue</strong></td>
<td>Ref</td>
</tr>
<tr>
<td>High frequency soap/ Low&lt;sup&gt;b&lt;/sup&gt; frequency cotton, cloth, tissue</td>
<td>0.66 (0.22, 2.00)</td>
</tr>
<tr>
<td>Low frequency soap/ High frequency cotton, cloth tissue</td>
<td>0.51 (0.26, 1.00)</td>
</tr>
<tr>
<td>Low frequency soap/ Low frequency cotton, cloth or tissue</td>
<td>0.66 (0.32, 1.35)</td>
</tr>
<tr>
<td>None</td>
<td>0.69 (0.18, 2.54)</td>
</tr>
</tbody>
</table>

<sup>a</sup>High frequency is defined as > 1/day
<sup>b</sup>Low frequency is defined as ≤ 1/day
Chapter 5: Validity and acceptability of self-collected samples for HPV testing in rural Malawi: Findings from a clinic-based feasibility study

5.1 Abstract

Background: The WHO recently endorsed HPV DNA testing as a first line cervical cancer screening method in countries without established screening programs. With the lowering costs of HPV testing, self-collection for HPV testing may be an effective way to expand screening to hard to reach women. 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 clinic in Lilongwe District, Malawi with genitourinary symptoms from January 2015-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 self-collection of samples.
Results: Among the 193 enrolled women, 22% had any hr-HPV. Comparing self- and clinician-collected samples for HPV testing, we found generally high agreement ($\kappa = 0.66$-$0.90$) 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 98% reporting willingness to do so again.

Conclusions: 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.

5.2 Introduction

Effective and widespread cervical cancer screening has greatly reduced cervical cancer incidence and related morbidity and mortality.[11] 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.[11] 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.[18,31] 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.[16,19,20]

DNA testing for human papillomavirus (HPV) offers an accurate alternative to VIA. HPV is a family of viruses that includes several oncogenic high-risk HPV types (hr-HPV). Two hr-HPV types, 16 and 18, are responsible for about 70% of all cases of cervical cancer.[134] The WHO recommends hr-HPV DNA testing as the primary cervical cancer screening approach in places where Pap testing has not been established.[134] 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.

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.[80,85–87] Self-collected samples have been shown to perform comparably to clinician-collected samples, but there is a wide range in agreement in published findings, suggesting that the population and method of collection or testing are important to consider when assessing the utility of self-collected samples.[32–35]

The recently lowered costs of HPV DNA testing may make this method of cervical cancer screening a viable screening option in a wide variety of settings. Combined with evidence that self-collection is a more effective way to screen women [25,81–83], 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 would 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.

5.3 Methods

5.3.1 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-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, abdominal pain, lower back pain, or any type of pelvic pain; incontinence or unusual urine odor, frequency or color; unusual vaginal discharge in terms of quantity, odor, color or consistency) was referred to study staff to assess eligibility. Women were eligible to participate if they were 18-49 years of age, spoke Chichewa, had at least one genitourinary symptom, consented to be examined and give biological specimens for testing, and resided in Lilongwe District. Women who were pregnant or menstruating were ineligible. Women provided written informed consent to participate.
5.3.2 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, Bedford, Massachusetts) and proceeded to perform a pelvic examination, which included VIA. The clinician used an endocervical brush to collect the sample for HPV testing. Following collection, he swirled the cervical brush in 20 ml of Preservcyt solution. Both clinician- and self-collected samples were stored at 4°C and were tested using the GeneXpert HPV assay at the end of the study (Cepheid, Sunnyvale, California).

The GeneXpert technology was developed to identify multi-drug resistant tuberculosis, and WHO supported the roll-out of GeneXpert systems for tuberculosis control programs in 21 countries throughout Africa and Asia.[15] While HPV testing using the GeneXpert is a new application of this technology, the GeneXpert platform is ubiquitous throughout Africa, including Malawi, and its use is supported by the Malawi Ministry of Health. The GeneXpert HPV test yields results in four categories: (a) negative for hr-HPV; (b) positive for HPV16; (c) positive for HPV18 or 45; or (d) positive for one of 11 pooled hr-HPV types (31, 33, 35, 39, 51, 52, 56, 58, 59, 66, and 68; Figure 6). The GeneXpert also includes quality assurance channels for sample adequacy control and a probe check control.
All results from VIA and HPV testing were recorded on paper forms and entered into an electronic spreadsheet by trained research staff.

Questionnaire. After the clinical exam, a research assistant 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 5-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 (Magpi, Washington, DC) and uploaded to an internet-based storage system daily.

Testing of clinician- and self-collected samples was not completed until the end of the research study and therefore we did not inform women if they had hr-HPV. However, women found to have hr-HPV are referred to VIA, and all participants in the research study underwent VIA as part of the research protocol. All women who were VIA-positive were referred to the district hospital for additional screening and treatment when necessary.

5.3.3 Data analysis

We first described the prevalence of hr-HPV in the study population, overall and by the four separate GeneXpert result categories for both self- and clinician-collected
samples. We then calculated the sensitivity and specificity of self-collected samples, using the clinician-collected samples as a reference standard. In order to assess agreement between the two sampling methods, we calculated a kappa statistic for overall hr-HPV type (positive for any hr-HPV vs. hr-HPV-negative) and by the 3 GeneXpert hr-HPV categories (HPV 16, HPV 18/45, additional hr-HPV types). As HPV testing is recommended only in women over 30 years of age due to the transient nature of HPV infections in younger women, we also conducted analyses restricting our sample to women over 30 years. We calculated the sensitivity, specificity and kappa agreement between self- and clinician-collected samples and VIA results. When calculating sensitivity and specificity for these analyses, we used VIA as the reference standard. Finally, using questionnaire data, we calculated frequencies to describe acceptability of self-collection among participants. All analyses were done using Stata 14.0 (StataCorp, College Station, TX).

5.3.4 Ethical approval

This project received ethical approval from the Ohio State University Institutional Review Board and the University of Malawi College of Medicine Research and Ethics Committee.
5.4 Results

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

We screened 234 women to enroll 200 in the parent study, 199 of whom consented to HPV testing. Test results are missing from 6 women and thus 193 are included in this analysis. Women without hr-HPV were slightly older than women with any hr-HPV (median age 34 vs. 31.5 years) and more likely to be married (98% vs. 79%; Table 8).

Among the women tested for HPV, 22% (n=42) had any type of hr-HPV by clinician-collected sample (Table 9). Six women were infected with multiple HPV types: one woman tested positive for HPV 16, HPV 18/45, and additional hr-HPV type(s), 4 women had both HPV 18/45 and additional hr-HPV type(s), and one woman had HPV16 and additional hr-HPV type(s).

5.4.2 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 18/45 (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, κ=0.73; Table 10). Overall compared to clinician-collected samples, self-collected samples were highly specific and varied in sensitivity by type of HPV (Table 10). Whereas specificity was 98-
100% depending on hr-HPV type, sensitivity was poorer; we found the highest sensitivity for HPV 18/45 at 91% (95% CI: 59%, 100%) and lowest sensitivity for HPV 16 at 50% (95% CI: 16%, 84%).

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 for detection of HPV 18/45 (Table 11). The exclusion of 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=0.76). 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.

5.4.3 VIA

Among the 193 women with available HPV results, 9% (n=16) had abnormal cervical lesions identified during VIA. Among these women, five (31%) also had hr-HPV using the clinician-collected samples and four (25%) using the self-collected samples (Table 9). Among the women with multiple categories of hr-HPV, none had abnormal cervical lesions. When comparing VIA to HPV testing, the agreement was poor (κ=0.06; Table 10). Using VIA as the reference standard, specificity was moderate (84%; 95% CI: 78%, 89%) while sensitivity was poor (25%; 95% CI: 7%,52%) Table 10). Excluding
women age 30 and under led to a slight increase in specificity and a slight decrease in sensitivity (Table 11).

5.4.4 Acceptability

Overall, women found the self-collection procedure easy to perform (98%), reported that the instructions were easy to understand (100%), and were confident they did it correctly (95%; Table 12). Most women reported they would recommend self-collection for HPV testing to a friend (99%) and more women preferred self-collection compared to clinician-collection sampling (61% vs. 39%). Three-quarters of women (74%) reported they would not have any concerns about self-collecting for HPV testing again in the future. Among those who expressed concerns, 12% reported worries that self-swabbing might hurt, 11% feared the HPV results following self-collection might not be accurate, and 7% were concerned they may not test correctly.

5.5 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, valid, and acceptable method of cervical cancer screening in this rural, Malawian population. The self-collection procedure was easy to incorporate into the clinic setting with all but one participant providing a sample, and all collected samples were sufficient for testing. High agreement between the self- and clinician-collected samples, and high levels of acceptability among participants, suggest
that self-collection procedures for HPV testing may be effectively incorporated into screening programs among rural, largely unscreened populations in Malawi.

Using a new HPV DNA test, we found high agreement in HPV test results between self- and clinician-collected samples, in line with previous research on other tests from a range of settings (kappa values ranging from 0.70-0.87). [65,70,72,74,76] Our findings also align with previous research that suggests agreement in HPV results from self- and clinician-collected samples can vary by HPV type. [72] 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 samples. [73]

A small number of women in our study, n=16, were found to have abnormal cervical lesions on VIA. Interestingly, we found that having hr-HPV was not significantly correlated with positive VIA, and among women with multiple types of hr-HPV detected, none had abnormal cervical lesions identified during VIA. Additionally, the majority of the women with positive VIA did not have hr-HPV. While we would not expect perfect agreement between VIA and HPV testing as they measure different markers we
anticipated that HPV testing would be a high sensitivity test and VIA would be more specific which the data do not support. We did not biopsy identified lesions, so it is possible that the observed abnormal lesions were caused by non-HPV agents. Other research found similar results: in a large study of 7,543 Chinese women, 6% were positive for VIA but negative for HPV compared to 10% of women with positive VIA and hr-HPV positivity. These findings in combination with our own suggest that VIA may capture non-HPV related cervical abnormalities and more research is needed to understand these findings[135]

Our findings suggest that self-collection of samples for HPV testing was widely acceptable, easy to perform and preferred to clinician-collection. 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 through community health workers were four times as likely to be screened for cervical cancer than women who were not offered the option to self-collect.[35] Additionally, restricting to the enrolled women who had not had a Pap test in the past four years, they were five times as likely to be screened than women in the control group, who were advised by health workers to seek cervical screening at health centers.[35] In a randomized control 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 cancer screening.[84]

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 experienced challenges explaining the self-collection procedure to women, suggesting that a future screening program must provide detailed instructions or have 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, [75]) or the collection and storage of dry samples, may perform comparably, [75,136] 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 population included younger women (under age 30) for whom HPV testing is not currently recommended. However, subgroup analyses with older women in the recommended range suggest that kappa of self- and clinician-collected samples and sensitivity and specificity findings were valid for both groups. 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 in women without genitourinary symptoms. Finally, VIA was performed by a single provider in the clinic, and we were not able to validate his findings with those of another provider, or to perform biopsy as a comparison to HPV testing and VIA. While biopsy data may have provided explanation for the discrepancy between VIA and HPV results in this sample, our study design using VIA without biopsy replicates typical clinical practice in this region and future research should examine the most appropriate screening method in this population.

While the rates of cervical cancer incidence and mortality have decreased precipitously in the last 40 years globally, the burden of disease falls disproportionately on women in low-resource settings without accessible screening programs. Self-collecting samples for HPV testing has the potential to eliminate many of the barriers of other cervical cancer screening programs that restrict access for women in low-resource settings. We submit that cervical cancer-screening programs using self-collected samples for HPV testing may be a feasible, valid, acceptable, and effective way to increase screening in hard-to-reach and under-screened populations.
Figure 6: Potential outcomes of GeneXpert HPV assay

- Samples will get a positive/negative result for each of the three outcomes.
Table 8: Participant characteristics of 193 care-seeking women by clinician-collected HPV results

<table>
<thead>
<tr>
<th></th>
<th>Any hr-HPV $^1$</th>
<th>No hr-HPV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 years</td>
<td>4 (9)</td>
<td>16 (11)</td>
</tr>
<tr>
<td>2-4 years</td>
<td>10 (24)</td>
<td>20 (13)</td>
</tr>
<tr>
<td>5-8 years</td>
<td>15 (36)</td>
<td>63 (42)</td>
</tr>
<tr>
<td>&gt;8 years</td>
<td>13 (31)</td>
<td>52 (34)</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>33 (79)</td>
<td>148 (98)</td>
</tr>
<tr>
<td>Single</td>
<td>9 (21)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Condom use last sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>4 (19)</td>
<td>12 (14)</td>
</tr>
<tr>
<td>No</td>
<td>17 (81)</td>
<td>74 (86)</td>
</tr>
<tr>
<td>Ever heard of cervical cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35 (85)</td>
<td>146 (97)</td>
</tr>
<tr>
<td>No</td>
<td>6 (15)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Age</td>
<td>31.5 (27, 36)</td>
<td>34 (29, 39)</td>
</tr>
<tr>
<td>Lifetime number of sexual partners</td>
<td>2 (2,3)</td>
<td>2 (1,2)</td>
</tr>
</tbody>
</table>
Table 9: HPV results, by collection modality

<table>
<thead>
<tr>
<th></th>
<th>Clinician</th>
<th>Self</th>
<th>Clinician</th>
<th>Self</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>None¹</td>
<td>151 (78)</td>
<td>161 (83)</td>
<td>12 (55)</td>
<td>11 (58)</td>
</tr>
<tr>
<td>Any hr-HPV</td>
<td>42 (22)</td>
<td>32 (17)</td>
<td>5 (23)</td>
<td>4 (21)</td>
</tr>
<tr>
<td>HPV 16</td>
<td>8 (4)</td>
<td>4 (2)</td>
<td>1 (4)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>HPV 18/45</td>
<td>11 (6)</td>
<td>11 (6)</td>
<td>1 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Additional hr-HPV</td>
<td>29 (15)</td>
<td>23 (12)</td>
<td>3 (14)</td>
<td>3 (16)</td>
</tr>
</tbody>
</table>

¹HPV results grouped by GeneXpert category.
Table 10: Concordance between HPV test results by self- and clinician-collected samples

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>(95% CI)</th>
<th>Specificity</th>
<th>(95% CI)</th>
<th>Kappa(^1)</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All types of hr-HPV</td>
<td>71</td>
<td>(55, 84)</td>
<td>99</td>
<td>(95, 100)</td>
<td>0.77</td>
<td>(0.65, 0.88)</td>
</tr>
<tr>
<td>HPV 16</td>
<td>50</td>
<td>(16, 84)</td>
<td>100</td>
<td>(98, 100)</td>
<td>0.66</td>
<td>(0.34, 0.97)</td>
</tr>
<tr>
<td>HPV 18/45</td>
<td>91</td>
<td>(59, 100)</td>
<td>99</td>
<td>(97, 100)</td>
<td>0.90</td>
<td>(0.77, 1.0)</td>
</tr>
<tr>
<td>Additional hr-HPV</td>
<td>69</td>
<td>(49, 85)</td>
<td>98</td>
<td>(95, 100)</td>
<td>0.73</td>
<td>(0.59, 0.88)</td>
</tr>
</tbody>
</table>

Concordance between VIA and HPV tests

<table>
<thead>
<tr>
<th>VIA/clinician collected hr-HPV</th>
<th>Sensitivity</th>
<th>(95% CI)</th>
<th>Specificity</th>
<th>(95% CI)</th>
<th>Kappa(^1)</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 (11, 59)</td>
<td>79 (72, 85)</td>
<td>0.06</td>
<td>(-0.08, 0.20)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

VIA/self-collected hr-HPV

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>(95% CI)</th>
<th>Specificity</th>
<th>(95% CI)</th>
<th>Kappa(^1)</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 (7, 52)</td>
<td>84 (78, 89)</td>
<td>0.06</td>
<td>(-0.09, 0.21)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Kappa measures expected vs. observed agreement
Table 11: Concordance between HPV test results by self- and clinician-collected samples in women older than 30 years of age

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Kappa$^1$ (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All types of hr-HPV</td>
<td>78 (56, 92)</td>
<td>99 (94, 100)</td>
<td>0.83 (0.69, 0.96)</td>
</tr>
<tr>
<td>HPV 16</td>
<td>75 (19, 99)</td>
<td>100 (97, 100)</td>
<td>0.85 (0.57, 1.0)</td>
</tr>
<tr>
<td>HPV 18/45</td>
<td>83 (36, 100)</td>
<td>99 (95, 100)</td>
<td>0.82 (0.59, 1.0)</td>
</tr>
<tr>
<td>Other hr-HPV</td>
<td>73 (45, 92)</td>
<td>98 (93, 100)</td>
<td>0.76 (0.57, 0.94)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concordance between VIA and HPV tests</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>VIA/ clinician collected hr-HPV</td>
<td>30 (7, 65)</td>
<td>82 (73, 88)</td>
<td>0.07 (-0.11, 0.26)</td>
</tr>
<tr>
<td>VIA/self-collected hr-HPV</td>
<td>20 (2, 56)</td>
<td>84 (76, 91)</td>
<td>0.03 (-0.15, 0.21)</td>
</tr>
</tbody>
</table>

$^1$Kappa measures expected vs. observed agreement
<table>
<thead>
<tr>
<th>Acceptability of self-collection</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very or somewhat easy to understand the self-collection instructions</td>
<td>191</td>
<td>(100)</td>
</tr>
<tr>
<td>Recommend self-collection to a friend</td>
<td>187</td>
<td>(99)</td>
</tr>
<tr>
<td>Very or somewhat easy to do self-collection of samples</td>
<td>188</td>
<td>(98)</td>
</tr>
<tr>
<td>Would use self-collection for HPV testing in the future</td>
<td>187</td>
<td>(98)</td>
</tr>
<tr>
<td>Very or somewhat certain self-collected correctly</td>
<td>181</td>
<td>(95)</td>
</tr>
<tr>
<td>Prefer self-collection over clinician-collection</td>
<td>114</td>
<td>(61)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concerns about self-collection</th>
<th>n</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No concerns</td>
<td>138</td>
<td>(74)</td>
</tr>
<tr>
<td>Might hurt</td>
<td>22</td>
<td>(12)</td>
</tr>
<tr>
<td>Might not be accurate</td>
<td>20</td>
<td>(11)</td>
</tr>
<tr>
<td>Might not do it correctly</td>
<td>14</td>
<td>(7)</td>
</tr>
</tbody>
</table>

1 Women could select multiple concerns
Chapter 6: Factors influencing Malawian women’s willingness to self-collect samples for HPV testing

6.1 Abstract

Background: Malawi has the highest incidence of cervical cancer in the world. Only 3% of Malawian women have ever been screened for cervical cancer. Self-collection of samples for human papillomavirus (HPV) testing could increase screening among underscreened and hard-to-reach populations. However, little is known about the acceptability of self-collection in rural African settings.

Aim: We aimed to characterize Malawian women’s willingness to self-collect vaginal samples for HPV testing and to identify potential barriers.

Design: We used data from the baseline wave of a community-based cohort study, collected from July 2014 – February 2015.

Setting: Participants were enrolled from the catchment area of a clinic in rural Lilongwe District, Malawi.

Methods: We enrolled women ages 15 - 39 years (n=824). Participants answered questions assessing willingness to self-collect a sample for HPV testing, concerns about testing and other hypothesized correlates of willingness to self-collect.

Results: Two-thirds of women (67%) reported willingness to self-collect a vaginal sample in their homes. Awareness of cervical cancer, supportive subjective norms,
perceived behavioral control, and clinician recommendations were all positively associated with increased willingness to self-collect samples for HPV testing. Identified barriers to self-testing endorsed by women included: concerns that the test might hurt (22%), that they might not do the test correctly (21%), and that the test might not be accurate (17%).

Conclusions: This study suggests that self-collection for HPV testing could be an acceptable cervical cancer screening method in this rural population. Findings identify modifiable beliefs and barriers that can inform the development of effective screening programs.

6.2 Introduction

Malawi has the highest age-adjusted incidence rate of cervical cancer in the world, at 76 cases per 100,000 women compared to an incidence rate of 43 cases per 100,000 women in eastern Africa overall and only 14 cases per 100,000 women globally.[15] Among those who are diagnosed with cervical cancer in Malawi, 60% will die from the disease.[4,15] In many countries, screening programs have successfully reduced the incidence and mortality of cervical cancer. However, despite rolling out a national cervical cancer screening program in Malawi using visual inspection with acetic acid (VIA) in 2004, access and utilization remains limited with only 3% of women ever screened for cervical cancer.[15] Cervical cancer screening programs are rare in Malawi and other low-resource regions for many reasons, including lack of health delivery infrastructure and trained personnel, limited health budgets, and competing healthcare
priorities.[18,31] With recent advancements in testing for human papillomavirus (HPV), which is responsible for nearly all cases of cervical cancer [137], the establishment of more accessible screening programs in conjunction with existing VIA programs is now possible.

HPV testing identifies presence of HPV infection using a clinician- or self-collected cervical or vaginal sample. HPV testing is now considered a complementary method in conjunction with a Pap test or even a first line screening method. The World Health Organization (WHO) recommends HPV testing as the primary method of cervical cancer screening in places where Pap testing has not been established.[134] Both self-collected and clinician-collected samples for HPV testing have been shown to have sensitivity and specificity comparable to Pap testing in identifying cervical intraepithelial neoplasia grade 2 or higher (CIN 2+).[17,138] HPV testing protocols also allow for a longer interval between screenings, as this approach detects disease progression earlier than cytology.[139] Importantly for an unscreened or under-screened population, one HPV test more effectively reduces cervical cancer incidence than one Pap test, potentially because of the higher sensitivity in detecting lesions with a high potential for malignant transformation.[140] For women who are considered at high risk of HPV infection (e.g. HIV-infected women or women who engage in sexual risk behaviors such as a higher number of partners [141,142]) or those who cannot access routine screening, self-collection of vaginal samples can lead to increased screening.[143]

While several studies have examined the validity and reliability of self-collected vs. clinician-collected samples for HPV testing [138], research on the acceptability of self-collected samples for HPV testing is much more limited. The existing data generally
suggests that women find self-collection acceptable and easy to perform.[90,91,93] We sought to examine the willingness of women in rural Malawi to self-collect a vaginal sample for HPV testing.

The theory of planned behavior (TPB) is a widely-used framework for understanding individual’s intentions and willingness to engage in health behaviors, including cancer screening and prevention behaviors.[103] Briefly, the TPB is comprised of three conceptual constructs: attitude toward the behavior, which considers behavioral beliefs and the individual’s evaluation of behavioral outcomes; subjective norms, which include how the individual perceives influential others’ opinions about the behavior and motivation to comply with those influential others; and perceived behavioral control which is the individual’s perceived power to engage in a particular behavior.[102,103] The TPB has been used to examine women’s intentions related to cervical cancer screening [105,107,144], but to our knowledge the present study is among the first to incorporate TPB concepts to understand the acceptability of self-collecting samples for HPV testing in a non-clinic, low-resource setting. We aimed to characterize Malawian women’s willingness to self-collect a vaginal sample for HPV testing and to identify the barriers that will need to be addressed before a cervical cancer screening program relying on self-collected samples can be successfully implemented.
6.3 Methods

6.3.1 Study design and population

This analysis used data from the baseline wave of a community-based cohort study on sexual and reproductive health decision making in rural Lilongwe District, Malawi from July 2014 to February 2015. The cohort study used two-stage, stratified, cluster sampling to select villages to enable enrollment of 1,000 women of reproductive age (aged 15-39 years). All women in the selected villages in the eligible age range were invited to participate. A subset of enrolled women received a series of questions on cervical cancer and cervical cancer screening (the questions went to a subset of participants because they were added to the survey after data collection began). Trained research assistants traveled to each selected village and conducted face-to-face interviews in Chichewa with all consenting women. Data were recorded on tablet computers using the Magpi electronic data capture system (Magpi, Washington, DC) and uploaded nightly to an internet-based storage system.

6.3.2 Measures

We used the TPB to develop survey questions related to women’s willingness to self-collect a vaginal sample for HPV testing in a non-clinic setting.

At the start of the series of questions, interviewers asked all women if they had ever heard of cervical cancer (yes/no). For those women who were not familiar with cervical cancer, interviewers explained that it “is a disease that attacks the cervix, which is part of the female reproductive system,” and then proceeded with the survey. Before
questions about self-collecting a vaginal sample for HPV testing, interviewers provided a brief description of the procedure indicating that: self-collection may help test for cervical cancer even if a woman doesn’t have symptoms; a woman could collect the sample by inserting a swab into the vagina; and that she could do this on her own at home, then give the sample to a health extension worker to take to the clinic for testing.

**Outcome.** Our primary outcome was women’s reported willingness to self-collect a vaginal sample for HPV testing in a non-clinic setting, if she were to be offered the opportunity (1=definitely not willing to 5=definitely willing) which we dichotomized into ‘willing’ (‘definitely willing’ and ‘probably willing’ responses) and ‘not willing’ (‘not sure,’ ‘probably not willing’ and ‘definitely not willing’) for analysis. To assess potential barriers to self-collection for HPV testing, interviewers also asked women what concerns they had about self-collection. For this item, research assistants did not prompt participants with possible options, but rather recorded all concerns for later analysis.

**Correlates.** Survey items asked each woman how serious she thought it would be if she had cervical cancer (1=not serious at all to 4=very serious); how worried she was about getting cervical cancer in the future (1=not at all worried to 4=very worried), and if she felt the test would protect her health (1=strongly disagree to 5= strongly agree). We assessed supportive subjective norms using a scale comprised of 2 items asking whether a participant thought her partner or other people important to her would approve of her self-collecting vaginal samples for HPV testing, if given the opportunity (1=strongly disagree to 5=strongly agree; α=0.90). We also asked if she would self-collect if a clinician recommended she do so (1=strongly disagree to 5=strongly agree). We assessed perceived behavioral control, with a scale composed of 3 agree-disagree items asking if a
participant: was confident that she could self-collect a vaginal sample correctly; confident that testing for cervical cancer at home could protect her health, and thought self-collection would be convenient (1=strongly disagree to 5=strongly agree; α=0.90).

The survey also assessed demographic, sexual health and behavioral factors that could influence women’s willingness to self-collect samples for HPV testing. Specifically, we included age, education, marital status, household income, lifetime number of sexual partners, and parity in the model.

6.3.3 Statistical analysis

We first ran descriptive statistics to assess the characteristics of study participants and women’s top concerns about self-collection of vaginal samples for HPV testing. We then ran separate unadjusted logistic regression models of the association between each independent variable of interest—age, education, marital status, household income, lifetime number of sexual partners, parity, awareness of cervical cancer, worry about cervical cancer, supportive subjective norm score, clinician recommendation, and perceived behavioral control score—and the binary willingness measure as the outcome of interest. Potential predictor variables that lacked variability in responses were not included in model building. We used backwards selection with a 0.05 significance level to develop our fully-adjusted, multivariable logistic regression model. Model fit was assessed using the Hosmer-Lemeshow goodness of fit test.[145] All analyses were conducted using Stata 12.0 (StataCorp, College Station, TX).
6.3.4 Ethical approval

This project received ethical approval from the Ohio State University Institutional Review Board and the University of Malawi College of Medicine Research and Ethics Committee.

6.4 Results

Of the 824 women who were offered the questions on cervical cancer and screening, 82% were married, 98% were HIV-uninfected by self-report, and 13% had less than 2 years of education (Table 13).

At the time of the survey, 85% had heard of cancer and 71% had heard of cervical cancer specifically. Nearly all women (93%) felt it would be very serious if they had cervical cancer, and 75% of women were moderately to very worried that they could get cervical cancer in the future (Table 14). Women generally felt self-collection could protect their health (mean 3.9±1.3; Table 14).

Most women (67%) reported being willing to self-collect a vaginal sample for HPV testing (Table 14). Sixty-two percent of women reported that they were definitely willing, and 5% reported they were probably willing. Twenty-four percent of women reported that they were definitely not willing to self-collect a vaginal sample for HPV testing. In unadjusted analyses, higher age, higher parity, awareness of cervical cancer before the survey, higher supportive subjective norm score, clinician recommendation for self-collection, and higher perceived behavioral control were all significantly associated with increased willingness to self-collect a sample for HPV testing (Table 15). After
adjustment, all effect estimates were attenuated. In the final multivariable model, women who were aware of cervical cancer had greater odds of being willing to self-collect a vaginal sample for HPV testing (OR 1.81; 95% CI: 1.25, 2.62), and those who perceived higher levels of supportive subjective norms had twice the odds (OR 2.00; 95% CI: 1.55, 2.59) of being willing to self-collect a sample for HPV testing (Table 15). Clinician recommendation (OR 1.34; 95% CI: 1.00,1.78) and higher perceived behavioral control (OR 1.90; 95% CI: 1.30,2.78) were also significantly associated with increased willingness to self-collect in the multivariable model.

When asked about their concerns about self-collection for future HPV testing, women’s most common response was that they did not have any concerns (42%). Women who did have concerns reported that they thought the test might hurt (22%), that they might not do the test correctly (21%), that the test might not be accurate (17%), and that they would rather go to a health facility (5%) than self-collect outside the clinic.

6.5 Discussion

Self-collection of vaginal samples for HPV testing is a novel approach to cervical cancer screening, with great potential to expand access to a broader population, particularly in low-resource settings. The present study provides insight into the acceptability of, and potential concerns about, this strategy among a sample of rural Malawian women, a population with elevated incidence of cervical cancer and associated mortality. We found that the majority of women reported being willing to self-collect a vaginal sample for testing in a non-clinic setting, and a plurality did not express any
concerns about the self-collection procedure. We also found that prior cervical cancer awareness, more supportive subjective norms, increased perceived behavioral control, and the weight of a clinician recommendation were all significantly associated with increased willingness. Future screening programs should consider these factors to maximize uptake and, consequently, impact. Additionally, programs relying on self-collection will need to address women’s concerns that the test might hurt, that the test may not be accurate, and provide detailed instructions so the woman is confident that she is doing it correctly.

Our finding that two-thirds of women report being willing to self-collect a vaginal sample for HPV testing is consistent with previous research. In other settings, when offered self-collection, women have found the procedure acceptable and report that they would be willing to do so again in the future. [21, 89–97] In one multi-site study, the majority of women in India and Uganda preferred self-collected vaginal sampling while women in Nicaragua equally preferred self-collection and clinician-collection of samples. [91] While two-thirds of women in our study reported being willing to self-collect a sample, women at all three of these previous studies had higher willingness than in our study. This may be due, at least in part, to greater comfort with the procedure when actually given the opportunity to test. [91] In another low-resource setting, among rural Thai women, 99.8% of women agreed to self-collect a vaginal sample for field-based HPV testing, 91% would self-collect in the future, and 96% would recommend the test to a friend. [21] Compared to our findings, in other settings where women were actually given the opportunity to self-collect, willingness and future intention to self-collect was even higher than what we found in our study.
While previous studies illustrate the acceptability of self-collection, the present study extends this work by identifying correlates of willingness that can be used to guide the development of future screening programs. Similar to other studies, we found that the TPB provided an effective framework to identify correlates of women’s willingness to self-collect a vaginal sample and that most demographic and sexual behavior variables were not significantly associated with women’s willingness to self-collect.[88,94,96,97,146] The factors we identified are informative for determining intervention points to increase utilization of screening programs. For example, based on our findings and similar to findings among Cameroonian and Mexican women [32,89], raising awareness of cervical cancer could lead to increased uptake of self-collected vaginal samples for HPV testing. Qualitative research among Malawian women found that cervical cancer knowledge was limited and thus this may be an important place to intervene to increase screening.[147] In line with our findings on the importance of perceived behavioral control, a study among urban Ugandan women that also utilized the TPB similarly found that perceived behavioral control was associated with increased willingness to self-collect, [33] thus screening programs may benefit from building women’s skills and confidence in their ability to self-collect samples.

Despite generally high acceptability of self-collection for HPV testing, we found that some women have concerns about the test and collection procedure. The top concerns found in the present study are consistent with findings from previous research in multiple settings suggesting that women may be concerned about pain or injury, [24,33,91] failing to self-collect an adequate sample, and the reliability or accuracy of a self-collected sample compared to one collected by a clinician. [24,91,98] Programs
implementing self-collected samples may be able to address these concerns. For example, future programs should emphasize that self-collection is as valid for HPV testing as clinician-collection.[138] Additionally, other research has found that self-collection procedures are more successful in programs in which community health workers were present [143] compared to those where women self-collected on their own [89], thus future programs may benefit from involving health workers to mitigate women’s concerns about correct collection procedures, and to answer questions that may arise.

Limitations of our study include our reliance on self-reported data, which is subject to social desirability bias. To minimize this bias we trained all research assistants prior to data collection. We also conducted a sensitivity analysis adjusting for interviewer and found it did not significantly change estimates. As we asked women about their willingness to self-collect a vaginal sample for HPV testing, rather than offering women the opportunity to self-collect samples, our findings may overstate actual self-collection behavior.[148] Future research should examine whether women will self-collect a sample when presented with an opportunity to do so. Our study was nested within a larger study (which was limited to women between the ages of 15-39 years), so our findings about willingness to self-test for HPV reflect the views of that population. HPV testing is recommended for an older population (≥30 years).[134] Currently in Malawi, HPV testing is neither the standard of care nor widely available. Thus it may be that by the time HPV testing were to be available, the women we interviewed will have reached the recommended age range for this screening method. Last, our study was conducted among women in single geographic area and may not be generalizable to women in other
regions, in more urban settings, or areas with established cervical cancer screening programs.

Our study provides important information about women’s willingness to self-collect vaginal samples for HPV testing, and identifies concerns that may impede successful implementation of future screening programs using this technology. Future research should assess actual self-collection in this population with the ultimate goal of implementing an accessible screening program and reducing the high incidence of and mortality from cervical cancer in this high-burden population.
Table 13: Participant characteristics among 824 women sampled in Malawi

<table>
<thead>
<tr>
<th>Variable</th>
<th>n(^{a})</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>672</td>
<td>(82)</td>
</tr>
<tr>
<td>Single</td>
<td>152</td>
<td>(18)</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 years</td>
<td>106</td>
<td>(13)</td>
</tr>
<tr>
<td>2-4 years</td>
<td>248</td>
<td>(30)</td>
</tr>
<tr>
<td>5-8 years</td>
<td>342</td>
<td>(41)</td>
</tr>
<tr>
<td>≥ Some secondary</td>
<td>128</td>
<td>(16)</td>
</tr>
<tr>
<td>Household income(^{a,b})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5,000 MWK</td>
<td>281</td>
<td>(38)</td>
</tr>
<tr>
<td>5,000-19,999 MWK</td>
<td>253</td>
<td>(34)</td>
</tr>
<tr>
<td>&gt;20,000 MWK</td>
<td>202</td>
<td>(27)</td>
</tr>
<tr>
<td>Age (median, IQR)</td>
<td>25</td>
<td>(20, 31)</td>
</tr>
<tr>
<td>Lifetime # of sexual partners</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 partners</td>
<td>70</td>
<td>(9)</td>
</tr>
<tr>
<td>1 partner</td>
<td>411</td>
<td>(50)</td>
</tr>
<tr>
<td>2 partners</td>
<td>210</td>
<td>(25)</td>
</tr>
<tr>
<td>≥ 3 partners</td>
<td>133</td>
<td>(16)</td>
</tr>
<tr>
<td>Parity (median, IQR)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV status (^{a,c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV +</td>
<td>15</td>
<td>(2)</td>
</tr>
<tr>
<td>HIV -</td>
<td>683</td>
<td>(98)</td>
</tr>
<tr>
<td>Abnormal genital discharge in the last 12 months(^{a,c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>101</td>
<td>(12)</td>
</tr>
<tr>
<td>No</td>
<td>722</td>
<td>(88)</td>
</tr>
<tr>
<td>Genital ulcers in the last 12 months(^{a,c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>69</td>
<td>(8)</td>
</tr>
<tr>
<td>No</td>
<td>753</td>
<td>(92)</td>
</tr>
<tr>
<td>Ever had an STI(^{a,c})</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>74</td>
<td>(9)</td>
</tr>
<tr>
<td>No</td>
<td>747</td>
<td>(91)</td>
</tr>
</tbody>
</table>

\(^{a}\) Some variables do not total to 824 due to missing data

\(^{b}\) 5000 MWK=US $11.37 during study enrollment

\(^{c}\) Based on self-report
Table 14: Attitudes towards cervical cancer and screening

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Awareness of cervical cancer</strong></td>
<td>582</td>
<td>71</td>
</tr>
<tr>
<td><strong>Willingness to self-collect samples</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Definitely willing</td>
<td>513</td>
<td>62</td>
</tr>
<tr>
<td>Probably willing</td>
<td>43</td>
<td>5</td>
</tr>
<tr>
<td>Not sure</td>
<td>30</td>
<td>4</td>
</tr>
<tr>
<td>Probably not willing</td>
<td>41</td>
<td>5</td>
</tr>
<tr>
<td>Definitely not willing</td>
<td>197</td>
<td>24</td>
</tr>
<tr>
<td><strong>Mean (SD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seriousness of cervical cancer (^a)</td>
<td>3.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Worry about cervical cancer (^a)</td>
<td>3.1</td>
<td>1.2</td>
</tr>
<tr>
<td>Subjective norms (^b)</td>
<td>3.6</td>
<td>1.4</td>
</tr>
<tr>
<td>People important to me approve of self-collection (^b,c)</td>
<td>3.6</td>
<td>1.4</td>
</tr>
<tr>
<td>My partner approves of self-collection (^b,c)</td>
<td>3.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Clinician recommendation (^b)</td>
<td>3.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Perceived behavioral control (^b)</td>
<td>3.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Self-collection can protect my health (^b,d)</td>
<td>3.9</td>
<td>1.3</td>
</tr>
<tr>
<td>Confident can perform self-collection correctly (^b,d)</td>
<td>3.4</td>
<td>1.5</td>
</tr>
<tr>
<td>Self-collection would be a convenient way to test for cancer (^b,d)</td>
<td>3.6</td>
<td>1.4</td>
</tr>
</tbody>
</table>

\(^a\) Range = 1-4  
\(^b\) Range = 1-5  
\(^c\) Item included in the subjective norms scale  
\(^d\) Item included in the perceived behavioral control scale
Table 15: Correlates of women's willingness to self-collect a vaginal sample for HPV testing

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.04 (1.02, 1.06)*</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 years</td>
<td>0.69 (0.41, 1.18)</td>
<td></td>
</tr>
<tr>
<td>2-4 years</td>
<td>0.71 (0.48, 1.04)</td>
<td></td>
</tr>
<tr>
<td>5-8 years</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>≥ Some secondary</td>
<td>1.07 (0.79, 1.45)</td>
<td></td>
</tr>
<tr>
<td>Relationship status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>1.20 (0.87, 1.64)</td>
<td></td>
</tr>
<tr>
<td>Household income&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5,000 MWK</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>5,000-19,999 MWK</td>
<td>1.06 (0.74, 1.51)</td>
<td></td>
</tr>
<tr>
<td>&gt;20,000 MWK</td>
<td>1.37 (0.95, 1.96)</td>
<td></td>
</tr>
<tr>
<td>Lifetime # of sexual partners</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 partners</td>
<td>0.76 (0.57, 1.03)</td>
<td></td>
</tr>
<tr>
<td>1 partner</td>
<td>Ref</td>
<td></td>
</tr>
<tr>
<td>2 partners</td>
<td>0.91 (0.62, 1.34)</td>
<td></td>
</tr>
<tr>
<td>≥ 3 partners</td>
<td>1.40 (1.02, 1.91)*</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>1.13 (1.04, 1.23)*</td>
<td></td>
</tr>
<tr>
<td>Awareness of cervical cancer</td>
<td>1.83 (1.36, 2.45)*</td>
<td>1.81 (1.25, 2.62)*</td>
</tr>
<tr>
<td>Worry about cervical cancer</td>
<td>1.12 (0.97, 1.29)</td>
<td></td>
</tr>
<tr>
<td>Subjective norms&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.34 (2.71, 4.12)*</td>
<td>2.00 (1.55, 2.59)*</td>
</tr>
<tr>
<td>Clinician recommendation</td>
<td>2.84 (2.31, 3.49)*</td>
<td>1.34 (1.00, 1.78)*</td>
</tr>
<tr>
<td>Perceived behavioral control&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.49 (2.61, 4.66)*</td>
<td>1.90 (1.30, 2.78)*</td>
</tr>
</tbody>
</table>

<sup>a</sup> 5000 MWK=US $11.37 at study enrollment

<sup>b</sup> Estimate reflects the OR for a 1 unit increase in the scale

* p<0.05

USD=United States dollar; MWK= Malawian kwacha
Chapter 7: Conclusions and implications for future research

7.1 Overview

While many countries have successfully reduced the incidence and mortality from cervical cancer, it remains a pressing health issue among Malawian women. This study provides important information on a potential risk factor for HPV and the implementation of screening methods to increase access to HPV screening for hard-to-reach women. We explored the association between highly prevalent intravaginal practices (IVP) and high-risk HPV (hr-HPV) and ultimately did not find an association between the two. However, our studies on IVP illustrated several gaps in knowledge about IVP, in particular measurement of IVP and the risk profile of women using multiple practices. In addition we explored expanding access to screening programs, focusing on a novel HPV DNA test using self-collected sampling. We found this screening method to be feasible and acceptable in our study population and have recommendations for roll-out to a more general population in the future.
7.2. Aim 1

7.2.1 Summary

Among the 193 women enrolled in the study, 96% reported using some type of IVP. We found that there was not a significant association between type, frequency, and a combined measure of frequency and type of IVP and prevalent hr-HPV.

7.2.2 Interpretation

While IVP are associated with other reproductive tract infections in other studies, we did not find any significant associations with type, frequency, or a combined measure of type and frequency of IVP with prevalent hr-HPV suggesting that IVP may not be a risk factor for prevalent hr-HPV. However, given the high reported prevalence of IVP in our study population, we may have been limited in our ability to detect significant differences.

7.2.3 Public health significance

As we did not find that IVP were associated with hr-HPV, other avenues should be explored for the prevention of hr-HPV infection. However, we did find a majority of the women in our study reported frequently performing IVP. IVP have been identified as risk factors for other genital tract infections, like bacterial vaginosis, and thus further research should examine if the IVP performed in this setting are associated with an increased risk of other sexually transmitted or reproductive tract infections.
7.2.4 Future research directions

A larger population or a population with more varied frequency of use of IVP should be studied to better estimate the association with hr-HPV as we were limited in our ability to make conclusions based on the high frequency and prevalence of IVP with few women included in the low frequency category. Additionally, we used cross-sectional data and could only make conclusions about prevalent hr-HPV. With more time points, conclusions could be made about the association between IVP and persistent HPV infections. Last, our study, like much previous research, is limited in the measurements of IVP. Future research should develop better metrics to assess IVP, such as biological measures. We also found that the metrics used to assess IVP are varied among different studies and when comparing effect estimates it would be beneficial for a standardized measure of IVP. IVP comprise a broad category of practices and it is important to differentiate the risks associated with the particular practices. Examining biological measures for IVP rather than self-reported practices would allow for a better understanding of these associations.

7.3 Aim 2

7.3.1 Summary

Among the 193 women enrolled in this study, 22% had a hr-HPV infection. Comparing self- and clinician-collected samples for HPV testing, we found there was generally high agreement (0.60-0.90) and high specificity (98%-100%) but varied sensitivity (50%-91%) for different subtypes of hr-HPV. We also found that self-
collection was acceptable, with 98% of women reporting it was easy to do and 98% reporting willingness to do so again.

7.3.2 Interpretation

Our study findings demonstrate that self-collection of samples for HPV testing is a feasible, valid, 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 procedures for HPV testing may be effectively incorporated into screening programs among rural, largely unscreened populations.

7.3.3 Public health significance

These findings suggest that this may be a feasible way to reduce cervical cancer incidence among rural Malawian women by increasing access to screening and catching HPV infection early in disease progression. This is also the first study to examine self-collection for HPV testing using the GeneXpert HPV assay. The GeneXpert system is ubiquitous throughout laboratories in sub-Saharan Africa after a roll-out sponsored by the WHO for TB testing. Establishing that self-collected samples are a valid method for this assay can expand access and allow for a lower cost method of HPV testing in low-resource settings where GeneXpert systems are already available.
7.3.4 Future research directions

The next step in this research program is implementation of an HPV testing screening program utilizing self-collected samples beyond the 200 women in our study. The short-term objective would be an assessment of the applicability of such a screening program in practice. The long-term objective is to determine if implementation can reduce incidence of cervical cancer in this population. Another line of inquiry stemming from this study is from the discrepancy in visual inspection with acetic acid (VIA) results and hr-HPV results with women testing positive for VIA but negative for hr-HPV. Further research is needed to understand the difference in the results and determine the best screening method for this population (VIA vs. HPV testing).

7.4 Aim 3

7.4.1 Summary

Two-thirds of women (67%) reported willingness to self-collect a vaginal sample for HPV testing in their homes. Awareness of cervical cancer, supportive subjective norms measured by approval of their partner or other important friends or family members, perceived behavioral control (as measured by confidence that she could self-collect a vaginal sample correctly; confidence that self-collection for HPV testing at home could protect her health, and belief that self-collection would be convenient) and clinician recommendations were all positively associated with increased willingness to self-collect samples for HPV testing. Identified barriers to self-testing endorsed by
women included: concerns that the test might hurt (22%), that they might not do the test correctly (21%), and that the test might not be accurate (17%).

7.4.2 Interpretation

This study suggests that self-collection for HPV testing could be an acceptable cervical cancer screening method in this rural population. Findings identify modifiable beliefs and barriers that can inform the development of effective screening programs.

7.4.3 Public health significance

Screening programs have successfully reduced the rates of cervical cancer in many countries. However, in Malawi the incidence and mortality rate of cervical cancer remains high. With the development of new advances in HPV DNA tests, the possibility of establishing new, more effective screening programs in conjunction with existing VIA testing can be realized. Self-collection of samples outside of the clinic can expand access to even more women by allowing for home- or village-based sampling under the guidance of community health workers. However, in order to make this screening program most successful, it is necessary to implement it in a way that is culturally appropriate and acceptable. Using the theory of planned behavior as a framework, we assessed individual attitudes towards HPV, cervical cancer and screening; social norms and influences that would inhibit or promote self-sampling for HPV testing, and potential barriers for self-sampling. This study provides information on the willingness of women
to self-collect a sample for HPV testing and to identify potential barriers that will need to be addressed before successful implementation.

7.4.4 Future research directions

As with aim 2, our findings suggest that next steps include a study of self-collection in this population with the ultimate goal of implementing an accessible screening program. Such a program could reduce the high incidence of and mortality from cervical cancer in this underscreened and high-risk population by increasing access to screening programs early on in disease progression.

7.5 Conclusions

The high prevalence of HPV and incidence of and mortality from cervical cancer among Malawian women is a major public health concern. While screening and early treatment has dramatically reduced the burden of cervical cancer in many countries, screening remains limited in Malawi with only 3% of women ever having a screening test. Research in other settings has highlighted co-factors that increase risk of hr-HPV infection and also the effectiveness of screening programs to reduce the disease burden of HPV. We provide information on the landscape of HPV infection in rural Malawian women, particularly the association between performing intravaginal practices and hr-HPV infection. We also provide information on the high frequency and types of IVP performed by these rural Malawian women. This project also provides information on the validity, feasibility, and acceptability of implementing a screening program using self-
collected samples for HPV testing. Identifying modifiable risk factors for HPV and establishing effective cervical cancer screening programs can reduce the high burden of cervical cancer in a setting with limited access to screening.
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121

122

123


Appendix A: BLT survey questions

IVP
My next questions are about practices some women do when they clean their genital area. For this question and future questions, when I say “inside the vagina,” I mean truly inside the vagina, not just around the labia (lips of the vagina) or vaginal opening.

1. In the past month how often did you clean inside your vagina with water only? Choose one response
   1. More than once a day
   2. Once a day
   3. A few times per week
   4. A few times per month
   5. Once a month or less often
   6. Never
   7. I don’t know
   8. I don’t want to answer

2. In the past month, how often did you clean inside your vagina with soap and water? Choose one response
   1. More than once a day
   2. Once a day
   3. A few times per week
   4. A few times per month
   5. Once a month or less often
   6. Never
   7. I don’t know
   8. I don’t want to answer

3. Next I’m going to ask you when you used soap and water to clean inside the vagina around the time of sex in the past month. Did you usually clean inside the vagina with soap and water before sex? After sex? Both times? Or neither time? Choose one response
   1. Before sex
   2. After sex
   3. Both times
   4. Neither time
5. I don’t know
6. I don’t want to answer

4. In the past month, how often did you clean inside your vagina with cotton, cloth, or tissue?
   Choose one response
   1. More than once a day
   2. Once a day
   3. A few times per week
   4. A few times per month
   5. Once a month or less often
   6. Never
   7. I don’t know
   8. I don’t want to answer

5. Next I’m going to ask you when you used cotton, cloth, or tissue to clean inside the vagina around the time of sex in the past month. Did you usually clean inside the vagina with cotton, cloth, or tissue before sex? After sex? Both times? Or neither time?
   Choose one response
   1. Before sex
   2. After sex
   3. Both times
   4. Neither time
   5. I don’t know
   6. I don’t want to answer

6. In the past month, how often did you insert any of these products inside your vagina for any reason: alum or other powder, herbs, leaves, castor oil, or any other vaginal product from a traditional healer or herbalist? You may have used these items before or during sex or at another time.
   Choose one response
   1. More than once a day
   2. Once a day
   3. A few times per week
   4. A few times per month
   5. Once a month or less often
   6. Never
   7. I don’t know
   8. I don’t want to answer

7. Next I’m going to ask you when you used any of these things to clean inside the vagina around the time of sex in the past month. Did you usually clean inside the vagina with any of these things before sex? After sex? Both times? Or neither time?
   Choose one response
1. Before sex
2. After sex
3. Both times
4. Neither time
5. I don’t know
6. I don’t want to answer
HPV questions
1. Was the participant offered the option to self-swab?
   Choose one response
   1. Yes
   2. No

2. Did the participant self-collect a swab?
   Choose one response
   1. Yes
   2. No

3. How easy was it for you to take the swab yourself?
   Choose one response
   1. Very easy
   2. Somewhat easy
   3. Neither easy nor difficult
   4. Somewhat difficult
   5. Very difficult
   6. I don't know
   7. I don't want to answer

4. How easy was it for you to understand the instructions for self-swabbing?
   Choose one response
   1. Very easy to understand
   2. Somewhat easy to understand
   3. Neither easy nor difficult to understand
   4. Somewhat difficult to understand
   5. Very difficult to understand
   6. I don't know
   7. I don't want to answer

5. How certain are you that you collected the swab correctly?
   Choose one response
   1. Very certain I did it correctly
   2. Somewhat certain I did it correctly
   3. Neither certain nor uncertain
   4. Somewhat uncertain that I did it correctly
   5. Very uncertain that I did it correctly
   6. I don't know
   7. I don't want to answer

6. In the future, would you be willing to take a self-swab again?
   Choose one response
   1. Yes
   2. No
3. Maybe
4. I don't know
5. I don't want to answer

7. Would you recommend self-swabbing to a friend?
Choose one response
1. Yes
2. No
3. I don't know
4. I don't want to answer

8. Do you prefer when the clinician collects the swab, or when you do it yourself?
Choose one response
1. Self
2. Clinician
3. No preference
4. I don't know
5. I don't want to answer

9. Would you have any concerns about self-collection of samples for HPV testing?
[DO NOT READ. Choose all that apply.]
Choose all that apply
1. It would be embarrassing
2. It might hurt
3. I might not do the test right
4. The test might show I have cervical cancer
5. The test might not be accurate
6. I would need my husband/partner’s approval to be tested
7. I am worried that HPV testing will make other people think I have cervical cancer
8. My religion/spiritual belief would not allow me to be tested
9. I do not think it is necessary to be screened for cervical cancer
10. Other, please specify
11. I would rather go to a health facility to get screened for cervical cancer
12. I wouldn't have any concerns
13. I don’t know
14. I don’t want to answer

10. Why not?
Appendix B: UTHA survey questions

1. Have you heard of cancer?
   1. Yes
   2. No
   3. I don’t know
   4. I don’t want to answer
   5. Not applicable

2. Have you ever heard of cervical cancer?
   1. Yes
   2. No
   3. I don’t know
   4. I don’t want to answer
   5. Not applicable

IF PARTICIPANT HAS NOT HEARD OF CERVICAL CANCER, START WITH:
Cervical cancer is a disease that attacks the cervix, which is part of the female reproductive system.

3. How serious do you think it would be if you had cervical cancer?
   1. Very serious
   2. Moderately serious
   3. A little serious
   4. Not at all serious
   5. I don’t know
   6. I don't want to answer

4. How worried are you that you could get cervical cancer in the future?
   1. Very worried
   2. Moderately worried
   3. A little worried
   4. Not at all worried
   5. I don’t know
   6. I don't want to answer
   7. Participant is a man
5. In some places, there is a home test that may help women test for cervical cancer, even if she doesn’t have symptoms. This test involves inserting a swab (like a Q-tip) into the vagina. A woman would do this test at home herself instead of going to the health care facility and then she would give the sample to the health surveillance assistant to take to the clinic for testing.

If this new type of test for cervical cancer were available, how willing would you be to use it?

1. Definitely willing
2. Probably willing
3. Not sure
4. Probably not willing
5. Definitely not willing
6. I don’t know
7. I don’t want to answer
8. Not applicable

6. What concerns would you have about testing at home for cervical cancer?
[DO NOT READ. Choose all that apply.]

Choose all that apply

1. It would be embarrassing
2. It might hurt
3. I might not do the test right
4. The test might show I have cervical cancer
5. The test might not be accurate
6. I would need my husband/partner’s approval to be tested
7. I am worried that HPV testing will make other people think I have cervical cancer
8. My religion/spiritual belief would not allow me to be tested
9. I do not think it is necessary to be screened for cervical cancer
10. Other, please specify
11. I would rather go to a health facility to get screened for cervical cancer
12. I wouldn’t have any concerns
13. I don’t know
14. I don’t want to answer

For the next series of questions, please say whether you strongly agree, agree, neither agree nor disagree, disagree, or strongly disagree.

7. Most people who are important to me would approve of me testing for cervical cancer at home if I am given the chance.

1. Strongly agree
2. Agree
3. Neither agree nor disagree
4. Disagree
5. Strongly disagree
6. I don’t know
7. I don’t want to answer

8. My partner would approve of me testing for cervical cancer at home if I am given the chance.
   1. Strongly agree
   2. Agree
   3. Neither agree nor disagree
   4. Disagree
   5. Strongly disagree
   6. I don’t know
   7. I don’t want to answer

9. I would test for cervical cancer at home if the health care provider suggested it.
   1. Strongly agree
   2. Agree
   3. Neither agree nor disagree
   4. Disagree
   5. Strongly disagree
   6. I don’t know
   7. I don’t want to answer

10. I am confident that testing for cervical cancer at home can protect my health.
    1. Strongly agree
    2. Agree
    3. Neither agree nor disagree
    4. Disagree
    5. Strongly disagree
    6. I don’t know
    7. I don’t want to answer

11. Testing for cervical cancer at home would be a convenient way to test for cancer
    1. Strongly agree
    2. Agree
    3. Neither agree nor disagree
    4. Disagree
    5. Strongly disagree
    6. I don’t know
    7. I don’t want to answer
<table>
<thead>
<tr>
<th>Variable of interest</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors associated with HPV</td>
<td>Condom use (Which methods have you or your partner ever used?)</td>
</tr>
<tr>
<td>Factors associated with HPV</td>
<td>Including the current partner, please can you tell me how many people you have had sex with in your life. Include your partners and all lovers you have had in your life, even if you were forced.</td>
</tr>
<tr>
<td>Healthcare utilization</td>
<td>What health facilities did you visit in the last 12 months?</td>
</tr>
<tr>
<td>Marital status</td>
<td>What is your marital status?</td>
</tr>
<tr>
<td>Education</td>
<td>How much school did you complete?</td>
</tr>
<tr>
<td>SES</td>
<td>If you think of all the sources of income your household had, coming into the household in the last month, how much money came in?</td>
</tr>
<tr>
<td>Age</td>
<td>What is your year of birth?</td>
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