Aus der Klinik für Gynäkologie mit Schwerpunkt gynäkologische Onkologie
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DISSERTATION

Feasibility of HPV-based cervical cancer screening in rural areas of developing countries with the example of the North Tongu District, Ghana.

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List of Abbreviations

ACCESSING	Adequate Cervical cancer Capacity building, Education and Screening by new Scientific INstruments in Ghana
BSGP	Broad Spectrum General Primer
CHPS	Community Health Planning and Services
CHW	Community Health Worker
CIN	Cervical Intraepithelial Neoplasia
DNA	Deoxyribonucleic Acid
GIZ	Gesellschaft für Internationale Zusammenarbeit GmbH
GRVD	German Rotary Voluntary Doctors
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus
HR	High Risk
NCDs	Non-Communicable Diseases
NGO	Non-Governmental Organization
LEEP	Loop Electrosurgical Excision Procedure
LMIC	Low and Middle Income Country
LR	Low Risk
LRS	Low-Resource Setting
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
SSA	Sub-Saharan Africa
STI	Sexually Transmitted Infection
USD	US Dollar
VIA	Visual Inspection with Acetic acid
WHO	World Health Organization

Summary

Abstract – English

Introduction

Cervical cancer gains increasing recognition as a preventable threat to women's health, as expressed by WHO Director General Dr. Ghebreyesus in his recent call for its elimination. Developing countries carry the global burden and despite existing recommendations for secondary prevention screening programs their implementation remains a barrier. This doctoral thesis aims to evaluate the feasibility of an HPV-based cervical cancer screening approach in the North Tongu District, Ghana.

Methods

This work studied (i) the methodological validity of self-sampling specimens from cervical cancer patients for HPV oncoprotein testing before its use in a screening population, (ii) the HPV prevalence among 2002 women, 18-65 years of age, in the general population of the North Tongu Disctrict, Ghana, through a cross-sectional population-based study with self-sampling collection in rural communities, and (iii) the natural history of HPV infection by longitudinal comparison of HPV type-specific persistence and clearance for 104 women over a four years' time period.

Results

Using self-sampling cervicovaginal lavage specimens for HPV oncoprotein detection was methodologically feasible with 95% sensitivity for HPV16/18 positive cervical cancer. However self-sampling cervicovaginal scraping specimens did not reveal reliable HPV oncoprotein test results during the cross-sectional assessment. The high-risk HPV prevalence found among women living in the North Tongu District, Ghana was 32.3% and 27.3% among women in the WHO-recommended screening age range of 30-49 years. Sample collection in the rural communities was successful. Infection-associated risk factors were (i) increasing age, (ii) increasing number of sexual partners and (iii) marital status, in particular not being married. Over the four years' time period 6.7% of the women observed had persistent high-risk HPV infection, while 93.3% cleared their initial infection and 21.2% acquired new infections.

Discussion

The high-risk HPV prevalence found among the general population and women 30-49 years is high and therefore requires careful planning and good infrastructure to triage high-risk HPV positive women and reduce the number of women needing treatment. Using HPV oncoprotein triage from the same self-collected specimen is not reliable at this point, stratification by sociodemographic factors risks stigmatization and retesting for HPV persistence necessitates a well-functioning recall system and HPV genotyping.

Conclusion

The high HPV prevalence found demands substantial governmental support and investment to build well-functioning screening infrastructure that offers necessary triage and treatment options for women high-risk HPV positive with increased risk for cervical cancer. Integrating local infrastructure and capacity is promising but requires regional assessment rather than one-size-fit-all approaches.

Abstract – Deutsch

Einleitung

Gebärmutterhalskrebs ist eine vermeidbare Bedrohung der Frauengesundheit weltweit, wie kürzlich von WHO Generalsekretär Dr. Ghebreyesus in seinem Aufruf zur Gebärmutterhalskrebseliminierung formuliert. Vor allem Entwicklungsländer tragen die Hauptkrankheitslast. Trotz zugänglicher Handreichungen zur Einführung von Ressourcen-adaptierten Präventionsprogrammen bleibt deren Umsetzung ein Hindernis. Ziel dieser Dissertation ist die Machbarkeit eines HPV-basierten Gebärmutterhalskrebspräventionsansatzes im North Tongu District, Ghana zu evaluieren.

Methoden

Diese Arbeit untersuchte (i) die methodische Validität selbstdurchzuführender Probennahme für die HPV Onkoproteindetektion basierend auf Proben von Gebärmutterhalskrebspatientinnen vor ihrem Einsatz im Screening, (ii) die HPV Prävalenz unter 2002 Frauen zwischen 18-65 Jahren der Allgemeinbevölkerung des North Tongu Distrikts, Ghana, im Rahmen einer populationsbasierten Querschnittsstudie unter Nutzung selbstdurchgeführter Probenentnahme in ländlichen Dörfern, und (iii) den Verlauf von HPV Infektionen durch eine Längsschnittuntersuchung der HPV Typ-spezifischen Persistenz und Ausheilung bei 104 Studienteilnehmerinnen über einen Zeitraum von vier Jahren.

Ergebnisse

Die HPV Onkoproteindetektion aus Proben selbstdurchzuführender Zervikalvaginalwaschungen war methodisch umsetzbar. Sie ermittelte HPV 16/18 bei positivem Gebärmutterhalskrebs mit einer 95%igen Sensitivität. Allerdings lieferten selbstentnommene Proben mittels einer Vaginalbürste, wie in der Querschnittsstudie durchgeführt, keine zuverlässigen HPV Onkoprotein Testergebnisse. Die ermittelte HPV Prävalenz karzinogener Genotypen im North Tongu Distrikt betrug 32,3% und in der WHO-empfohlenen Altersgruppe zur Früherkennung 30-49 jähriger Frauen 27,3%. In den ländlichen Dörfern selbstdurchgeführte Probenentnahme verlief erfolgreich. Mit einer HPV Infektion assoziierte Risikofaktoren waren (i) ansteigendes Alter, (ii) zunehmende Anzahl von Sexualpartnern und (iii) der Familienstand, vor allem nicht

verheiratet zu sein. Die Längsschnittuntersuchung zeigte persistierende HPV Infektionen karzinogener Typen bei 6,7% der Frauen und Ausheilung bei 93,3% der Frauen über einen Zeitraum von vier Jahren, wobei 21,2% neue Infektionen akquirierten.

Diskussion

Die hohe HPV Prävalenz karzinogener Typen bei Frauen der Allgemeinbevölkerung und 30-49 jährigen Frauen erfordert eine sorgfältige Planung und gute Infrastruktur, um die Vielzahl HPV-positiv getesteter Frauen triagieren und somit die Anzahl der Frauen, welche tatsächlich eine weitere Behandlung benötigen, reduzierten zu können. Triage mittels HPV Onkoproteindetektion basierend auf derselben Probe ist zu diesem Zeitpunkt noch keine zuverlässige Option. Eine Risikostratifizierung anhand soziodemographischer Kriterien birgt das Risiko von Stigmatisierung und wiederholte Testung zur Detektion persistierender HPV Infektionen erfordert ein zuverlässiges Rückrufsystem und HPV Genotypisierung.

Schlussfolgerung

Die hohe ermittelte HPV Prävalenz erfordert erhebliche Unterstützung und Investition seitens der Regierung um eine funktionstüchtige Präventionsinfrastruktur aufzubauen, welche die benötigten Optionen zur Triage und Behandlung HPV-positiv getesteter Frauen bereitstellen kann. Dabei ist es erfolgsversprechend die lokale Infrastruktur und Kapazität zu nutzen, was eine regionale Prüfung statt die Anwendung allgemeingültiger Modelle erfordert.

1. Introduction

1.1 Global situation HPV & cervical cancer

Cervical cancer is the 3rd most common cancer among women worldwide leading to approximately 570,000 new cancer cases and 311,000 cancer deaths annually (1). About 99% of cervical cancer cases are caused by persistent infection with human papillomaviruses (HPV) (2) and particularly the genotypes 16 and 18 cause about 70% of the global burden (3). Additional HPV types classified as oncogenic (from hereon called high-risk/HR-HPV) by the World Health Organization (WHO) are namely 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (4). Additional risk factors have been identified to contribute to the transition risk of persistent HPV infection to malignant cervical lesions, which are smoking, high parity, long-time use of oral contraceptives as well as infection with other sexually transmitted infections (STIs) (5).

Developing countries especially carry the global burden of cervical cancer with about 76% of incident cases and 80% of annual deaths occurring in Africa and Asia (1). Reasons for this are poorly developed health systems, lack of financial and technical resources as well as human capacity to diagnose and treat cervical cancer and partially also lack of awareness (6).This high rate of cervical cancer incidence is projected to increase by 90% until 2030 in Sub-Saharan Africa (SSA), when considering the current increase in incidence, aging, and population growth (7). Factors such as the increasing number of human immunodeficiency virus-positive (HIV) patients further contribute to this projection (7).

1.2 HPV & cervical cancer in Ghana

In Ghana, the crude incidence rate of cervical cancer is about 19 per 100,000 women annually, making it the 2nd most common female cancer among women at the age of 15-44 years and the 2nd leading cause of cancer deaths (8). Poor screening coverage and late presentation at the clinic contribute to this high incidence (9, 10). Furthermore, data investigating HPV prevalence, the causal factor for cervical cancer, in the general population in Ghana is very rare and limited to small studies or specialized populations. HPV prevalence rates stated are for example 11% among 75 women attending the gynaecology outpatient clinic in Accra (11), or 42% and 77% among 100 HIV negative and 107 HIV positive women from Kumasi, respectively (12).

There is currently no national program for cervical cancer screening in Ghana. Although the national control program for non-communicable diseases (NCDs) states the aim of implementing a cervical cancer screening system at least on a regional level as a national policy priority, the actual outline of a screening program remains vague and little of the suggested means have been implemented until today (13, 14).

1.3 Cervical cancer prevention and current perspectives in Sub-Saharan Africa

Cervical cancer can be prevented at different stages. For primary prevention, the WHO recommends, among other measures, vaccination of girls aged 9-13 years, before the debut of sexual activity (15). Since the introduction of the first vaccines in 2007, significant reductions of HPV16 and 18 prevalence have been reported for countries with female vaccination coverage of ≥50% (16). Decrease of HPV31, 33, 45 prevalence in girls even suggests cross-protection, and reduction of anogenital warts in girls 13-19 years and boys <20 years indicates herd immunity (16). Despite this success it is estimated that only 1% of the so far implemented vaccination programs were in low- or lower-middle-income countries (LMICs) and that only 3% of women aged 10-20 years in less developed regions received the full course of vaccine by 2014 (17). Until wider and more efficient vaccination coverage can be achieved, secondary prevention targeting the screening and treatment of premalignant lesions, resulting from persistent infection with HR-HPV, is essential.

While in high-income countries secondary prevention through screening programs for cervical cancer precursors using cytology have prevented about 80% of the projected deaths (18), hardly any organized screening programs exist in SSA. In developing countries, cytology is not feasible as a population-based screening method due to the complex and expensive nature of such screening programs that require frequently repeating screening intervals and a well-functioning health infrastructure (19). Therefore, the WHO recommends in their current guidelines population screening using visual inspection with acetic acid (VIA), or if resources permit HR-HPV testing for women aged 30-49 years to detect cancer precursors (15). Preferably screening should be conducted in a screen-and-treat approach to avoid high rates of patients lost to follow-up (15).

Although VIA can be used in a screen-and-treat approach and has for example been implemented as the national screening strategy in many parts of Zambia (20), its effect

on successfully reducing cervical cancer mortality is under debate. A study from India has shown that a single round of HPV testing followed by respective treatment significantly reduced the detection of advanced cancer and the number of deaths, while VIA did not achieve this effect (21). Especially in low-resource settings (LRS), where already a single round of screening is a great challenge for the health system, these results are highly important.

1.4 Self-sampling and HPV oncoprotein testing - novel tools aiding secondary prevention

Further challenges for screening programs in LRS are to accomplish a high coverage among the population at risk and the management, meaning triage and treatment, of screen-positive women (22).

Recent developments include the introduction of self-sampling for HPV-based screening. It allows women to self-collect samples, possibly even at their own home. Studies have shown that self-sampling is well accepted by women and even increases cervical cancer screening uptake among non-responders (23). The accuracy of HPV testing from self-collected samples compared to physician-obtained samples is similar when using polymerase chain reaction (PCR) assays (24). Using self-sampling devices is a highly promising approach for cervical cancer screening in developing countries, as it allows decentralization from the hospital and screening on the doorsteps of the women, which can substantially increase the population coverage of screening programs.

Due to the concerns that highly sensitive HPV-based screening, using HPV DNA or RNA, could result in a high number of women in need for follow-up before treatment, various triage methods with higher specificity for true disease, among those HPV oncoprotein testing, are being discussed (25). HPV oncoprotein testing is based on the principle of detecting HPV oncoproteins, which are characteristically synthesized during proliferation of cervical lesions and a prerequisite for the development of cervical cancer (26). A study in China has postulated that based on the high specificity for cervical intraepithelial neoplasia (CIN) grade 3 or higher and the reduced number of colposcopy referrals, HPV oncoprotein testing is a good triage method and could even be considered for cervical cancer screening (27).

1.5 Objective and research questions

While a lot of evidence on HPV-based screening, its cost-effectiveness, the acceptability of self-sampling, and potentially useful triage of positive screens, to mention just a few of the above presented secondary prevention aspects, have been published, implementation of population-wide screening remains a challenge in regions with scarce resources and mostly donor- and non-governmental organization-driven (NGO) prevention programs (28). Therefore, the aim of this work was to determine the potential feasibility of an HPV-based screening approach (including HPV DNA and oncoprotein testing) using self-sampling for secondary cervical cancer prevention, which is integrated in the existing public health system in Ghana.

The research question framing this work was: Is HPV-based cervical cancer screening feasible in rural areas of developing countries with the example of Ghana?

In detail, this work was divided into the following secondary research questions:

1) Is self-sampling a valid method for HPV oncoprotein detection?
2) What is the HPV prevalence in rural Ghana and what are the implications for screening?
3) What influence could the natural history of HPV and cervical cancer have on the screening strategy?

2. Methods

2.1 The ACCESSING study

ACCESSING is the acronym for “Adequate Cervical cancer Capacity building, Education and Screening by new Scientific INstruments in Ghana” and was a program funded by the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) and the German Rotary Voluntary Doctors (GRVD). The program was conducted during a hospital partnership in collaboration between the Catholic Hospital Battor in the Volta Region, Ghana, and the Charité-Universitätsmedizin Berlin, Clinic for Gynecology, Germany. The aims of this program were to assess the HPV prevalence with a cross-sectional study design in the general population, to evaluate a potential screening algorithm for cervical cancer screening on its feasibility, and to build the capacity to independently and autonomously introduce cervical cancer screening. The program was conducted from October 2013 until February 2017 and included two pilot studies with a total of 400 women screened at the Catholic Hospital Battor as well as a main study with 2002 women screened from the rural and urban communities in the North Tongu District. The screening in the communities was supported by the local Community Health Planning and Services (CHPS) system with community health workers (CHWs) as community members providing basic health and medical care. In this study CHWs recruited participants and supported sample collection. This doctoral thesis summarizes the results from the ACCESSING study and its preceding work. Ethical clearance for this study was given by the Ghana Health Service Ethical Review Committee (Ref. No. GHD-ERC: 05/05/13) in October 2013.

2.2 Study population

Depending on the research question in focus and phase of the ACCESSING study, the study populations differed from each other. This is also reflected by the different publications resulting from this study, each one focused on a different aim and study population, as listed below:

1) The methodological validation of self-sampling for HPV oncoprotein testing focused on a study population of confirmed HPV16/18 positive cervical cancer patients from the clinic of gynaecology at Charité – Universitätsmedizin Berlin, who have not yet received any kind of treatment. This group consisted of 20 patients that were

selected in consecutive order based on their patient record between January 2013 and July 2014.

2) HPV prevalence analysis was based on the ACCESSING main study, a cross-sectional study including 2002 women from the North Tongu District that were sampled to represent the number of women living in the respective community (based on the 2010 Population Census).
3) Longitudinal analysis on natural history of HPV included women from the ACCESSING pilot study. From the 400 women screened during the pilot study phase 104 women were included in the analyses. These women had already been screened for HPV in 2010/2011 during a previous study conducted by the Catholic Hospital Battor in collaboration with the University of Accra.

2.3 Sample types

Depending on the study (methodological validation, cross-sectional study, or longitudinal study) different types of samples were collected from the participating women, as shown in Table 1.

Table 1: Overview of sample types, collection and processing methods used.

Sample type	Collection	Processing	Storage
Cotton swab (cervical)	Physician or nurse-collected	Kept dry	-20°C
Cytobrush Berlin (cervical)	Physician or nurse-collected	Kept dry	-20°C
Cytobrush Ghana (cervical)	Physician or nurse-collected	Immediately washed in 20 ml PreservCyt solution; Aliquots of 2 ml used for DNA extraction	4°C
Delphi Screener (vaginal lavage)	Self-collected	Immediately collected in 50 ml Falcon tubes	-20°C
Evalyn brush (vaginal scraping)	Self-collected	Within 7 days; Brushes soaked overnight and washed in 1 ml PreservCyt solution to retrieve cell suspension; Aliquots of 100 µl cell suspension used for DNA extraction	Aliquot: -20°C, Remaining sample: 4°C

These included physician or nurse-collected cotton swab samples, cytobrush samples and/or self-collected samples using either the Delphi Screener or the Evalyn brush (both Rovers Medical Devices, The Netherlands). The exact sample types and processing methods used for each study are stated in the respective publication.

2.4 DNA extraction

Two different methods for deoxyribonucleic acid (DNA) extraction were used as part of this thesis. For the method-validation and the longitudinal analysis DNA extraction was done using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. For the cross-sectional HPV prevalence analysis DNA was extracted using the Maxwell® 16 LEV Blood DNA Kit (Promega, Madison, USA) according to manufacturer's instructions.

2.5 HPV genotyping

For HPV genotyping 5 µl of the extracted DNA was used for broad spectrum general primer (BSGP) 5+/6+ PCR followed by Luminex readout. This method was performed according to Schmitt et al. 2008 (29). According to WHO monograph classification HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were considered as HR-HPV genotypes. HPV66 and 68a and 68b were defined as probable, HPV26, 53, 73, and 82 as potential HR-HPV and the HPV types 6, 11, 42, 43, 54, 57, 70, 72, 90 as low risk types (LR-HPV) (4).

2.6 HPV oncoprotein testing

The OncoE6 Cervical Test (Arbor Vita Corporation, Fremont, CA, USA) was used according to manufacturer's instructions for detection and differentiation of HPV16/18 oncoprotein during the methodological validation of self-sampling. All three collected sample types (vaginal lavage, cotton swab, and cytobrush) were tested as described in the publication "Performance of OncoE6 cervical test with collection methods enabling self-sampling" by Krings et al., BMC Womens Health. 2018;18(1):68 (30). During the cross-sectional study self-collected vaginal scraping specimens were tested with the OncoE6 Cervical Test according to manufacturer's instructions.

2.7 Clinical follow-up

All women participating in the cross-sectional or longitudinal part of the ACCESSING study and tested HR-HPV positive were invited for clinical follow up free of charge at the

Catholic Hospital Battor, Ghana. During the longitudinal study women testing positive were immediately referred for colposcopy, while women testing positive during the cross-sectional study were first recalled for cytology and then based on cytology results referred for colposcopy. Based on the diagnosis by the treating gynaecologist, further treatment via loop electrosurgical excision procedure (LEEP) or hysterectomy was offered. Definitive histology was obtained from local pathologists. Women participating in the method-validation study part were already under treatment at Charité – Universitätsmedizin Berlin, Germany

2.8 Statistical analysis

Statistical methods were used as part of the cross-sectional study to assess sociodemographic risk factors as categorical variables for HR-HPV infection as the dependent variable (31). For this univariable and multivariable logistic regression were conducted. A stepwise forward selection of variables was chosen using the likelihood ratio test to compare fit of the models. The analysis was performed with STATA version 15 (StataCorp LLC, College Station, Texas, USA).

3. Results

In order to answer the question “Is HPV-based cervical cancer screening feasible in rural areas of developing countries with the example of Ghana?” this doctoral thesis was divided into three parts, based on the secondary research questions stated above and the three publications summarized here. While the first part investigated aspects of technical validity, the following parts were focused on the aspects of epidemiology of HPV in Ghana, and the natural history from HPV infection to cervical cancer progression.

3.1 Technical validation of self-sampling-based HPV oncoprotein testing (30)

The technical validation of HPV oncoprotein testing, primarily for vaginal-lavage samples collected with a self-collection device, showed concordant test outcomes by the OncoE6 Cervical test with HPV genotyping results. In 18 out of 20 cervical cancer patients, the results obtained from the antibody-based OncoE6 Cervical test for vaginal lavage samples correctly corresponded with the respective HPV16 or 18 positivity, as determined by BSGP5+/6+ genotyping. The oncoprotein test results obtained from the vaginal lavage (self-collection device) were compared to the results from the manufacturer’s recommended swab sample and the cytobrush sample, which was also used as a reference result for HPV genotyping.

Two patient sample sets with discordant results were further analysed. For one patient all three samples collected were found to be negative for the OncoE6 Cervical test but HPV16 positive for the reference genotyping. The reference cytobrush sample was sent for sequencing and revealed a mutation influencing the protein sequence of the E6 protein that lies within the binding region of the OncoE6 Cervical test antibody. It was concluded that this could be the reason for the false negative results seen in all three samples. The second patient with discordant results showed negative oncoprotein results with the cytobrush as well as the vaginal lavage sample, while the manufacturer’s recommended cotton swab sample was positive. The cellular content of the samples was evaluated and the low cell density in the two negative samples was considered as a plausible reason for these false negative results.

Overall and after exclusion of the sample with E6 epitope mutation, the sensitivity of the vaginal lavage from a self-collection device and the cytobrush sample for HPV16/18

oncoprotein detection was 95%, respectively. Sensitivity for the swab sample was 100%. Specificity could not be calculated, as no HPV16/18 negative patients were included in the study set-up. The detailed results are presented in the publication "Performance of OncoE6 cervical test with collection methods enabling self-sampling" by Krings et al., BMC Women's Health. 2018;18(1):68 (30).

3.2 HPV prevalence results from the North Tongu District, Ghana (31)

As part of the cross-sectional study women from rural communities of the North Tongu District were recruited through the local community health worker system and invited for HPV-based cervical cancer screening. The 2002 participating women received a self-sampling brush (Evalyn brush, Rovers) to self-collect their vaginal sample and send it to the Catholic Hospital Battor for testing. In total, 1943 HPV test results were valid and constitute the HPV prevalence presented here.

Among the 1943 women with valid HPV test results, the average age was 32 years, ranging from 18 to 65 years. The majority of women had completed Junior High School or higher (59.8%), about 42.2% of the women were married and 38.0% had 1-2 children (range: 0-13 children), 59.2% had 2 or more sexual partners and 51.3% were 18 years or younger at their first sexual intercourse.

The HR-HPV prevalence found in the North Tongu District in Ghana was 32.3% (95% CI: 30.2-34.5) with 9.7% (95%CI: 8.4-11.1) being positive for multiple HR-HPV types. The prevalence of LR-HPV as single or multiple infections was 18.4% (95%CI: 17.7-20.2). In total, 53.5% (95%CI: 51.3-55.8) of the women were HPV negative for any genotype. The most prevalent HR-HPV types in descending order were HPV16 (7.4%; 95% CI: 6.3-8.7), HPV52 (7.2%; 95% CI: 6.1-8.5), HPV35 (4.8%; 95%CI: 3.9-5.8), HPV59 (4.7% 95% CI: 3.8-5.8) and HPV56 (3.9%; 95% CI: 3.1-4.8). Overall, 12.4% of the women were positive for the HR-HPV types 35, 56 and 59, which are not covered by any of the currently available HPV vaccines.

The age-specific HPV prevalence was highest among women younger than 25 years, reducing during the age of 25-54 years and showed a second peak for women above 54 years of age. Overall, the HR-HPV prevalence was 27.3% among women of the WHO-recommended screening age range from 30-49 years. Factors associated with HR-HPV infection, resulting from multivariable logistic regression analysis, were: (i) increasing

age, (ii) having any sexual partner, while the odds for infection increased with an increasing number of sexual partners and (iii) marital status, in particular not being married. The detailed results are presented in the publication “Characterization of Human Papillomavirus prevalence and risk factors to guide cervical cancer screening in the North Tongu District, Ghana“ by Krings et al., PLoS One. 2019;14(6) (31).

The results from the HPV oncoprotein testing were invalid and could therefore not be analysed. Attempts to investigate reasons for these invalid test results remained inconclusive, despite repeat testing of samples and troubleshooting together with the company providing the test kits.

3.3 Longitudinal observations on HPV (32)

Among 104 women from the North Tongu District, HPV screening was conducted in 2010/2011, repeated in 2014 and HPV genotyping results compared for clearance, re-infection, and persistence of HPV infections.

Interestingly, 76.9% (80/104) of the women were HR-HPV negative and had completely cleared their HR-HPV infection in 2014. When comparing the respective HR-HPV genotypes among women who tested positive in 2010/2011 and 2014, only 6.7% (7/104) women were still positive for the same HR-HPV type in 2014 as they were in 2010/2011. Overall, 21.2% (22/104) of the women got re-infected, meaning they had cleared the original infection and were re-infected with a new and different HPV type. This resulted in an overall clearance rate from the initial HR-HPV type of 93.3% (97/104) for a time period of up to four years and a persistence rate of 6.7% (7/104).

Out of the seven women with persistent HPV infection, one woman was diagnosed with CIN2 and one woman with invasive cervical cancer in 2014. The woman with CIN2 was persistent for HPV68 and the woman with invasive cervical cancer persistent for HPV16. Another woman suspicious of cancer from colposcopic examination was lost to follow-up. Out of the 22 women re-infected with a new HR-HPV genotype one case of CIN2 was detected.

The detailed results are presented in the publication “Dynamics of genotype-specific HPV clearance and reinfection in rural Ghana may compromise HPV screening approaches.” by Krings et al. Papillomavirus Res. 2019;7:45-51 (32).

4. Discussion

4.1 Is self-sampling a valid method for HPV oncoprotein detection?

The results of this methodological validation suggest that the lavage samples obtained with the self-sampling device Delphi Screener are in principle technically compatible to be used in conjunction with the OncoE6 Cervical Test and that this combination had a high sensitivity (95%) for the detection of cervical cancer (30). In order to answer whether self-sampling is a valid method for HPV oncoprotein detection, a further investigation of this approach in a larger population-based study is needed to assess its sensitivity. This is necessary since (i) one case of cervical cancer was not detected by this suggested method combination and (ii) the OncoE6 Cervical Test only detects HPV16 or 18 positive cases of cervical (pre)cancer.

Unfortunately, during the course of the ACCESSING study, the company originally supplying the Delphi Screener device declared insolvency and therefore, a different self-sampling device (Evalyn brush) needed to be used for the suggested assessment during the cross-sectional study part that included screening 2002 women in the North Tongu District, Ghana (see 2.3). Despite a successful pilot phase comparing Delphi Screener and Evalyn brush, the results of the assessment during the cross-sectional study were generally invalid (see 3.2).

A study conducted in Brazil, showed that HPV oncoprotein testing performed on self-sampling brush samples (Evalyn brush) was overall feasible and had a sensitivity of 66% for HPV16/18 positive CIN3+ cases. One case of HPV18 positive adenosquamous tumour was missed by HPV oncoprotein testing but detected via the positive HPV DNA result followed by colposcopy (33).

While there is an urgent need and difficulty for reliable triage and follow-up of all HR-HPV positive screened women in LRS (22), the OncoE6 Cervical test demonstrated promising specificity and positive predictive value (PPV) for this purpose with physician collected specimens (34). Preferably using the same sample collected for HPV DNA-based screening also for the HPV oncoprotein triage could limit the number of visits needed for patients. This could bring algorithms closer to screen-and-treat approaches. However, the missed cases of cervical cancer in our methodological validation study and the Brazil publication (30, 33), combined with the inconclusive test results during

the cross-sectional study, prevent us recommending a triage combination with the Evalyn brush self-sampling device in conjunction with HPV oncoprotein testing at this point and further research is needed.

4.2 What is the HPV prevalence in rural Ghana and what are the implications for screening?

The 32.3% HR-HPV prevalence found in this cross-sectional study for the general population of the North Tongu District, Ghana was remarkably higher than the expected and WHO-stated prevalence of 21.3% in Western Africa (31). This also holds true for the WHO screening-recommended women aged 30-49 with an HR-HPV prevalence of 27.3%. 12.4% were positive for HR-HPV types not covered by any vaccine and therefore without any protection even if the nonavalent vaccine is introduced nationally. Additionally, 9.7% of the women had multiple HR-HPV infections, an important factor for cervical cancer prevention, as multiple infections persist longer and could therefore lead to a higher chance of developing cervical cancer (35). These screening results highlight the urgent need for secondary cervical prevention and the implementation of screening in Ghana.

Simultaneously, the need to follow-up >25% of the women due to their HR-HPV status but still unclear disease status can easily overburden the health system. Though it is advantageous to use HPV testing as a highly sensitive primary screening method, it requires realistic and careful planning of an adequate triage, follow-up and treatment strategy to effectively protect HR-HPV positive women detected from developing cervical cancer.

Based on the results of this cross-sectional study it may be worthwhile to consider prioritizing certain women at increased risk for screening in order to reduce the number of women to be screened and in need of follow-up (i.e. women with multiple HR-HPV infections). However, this would limit the types of HPV tests that can be used, as it requires an HPV test that can detect multiple infections. Selecting certain sociodemographic high-risk groups for screening, such as women with multiple sexual partners or unmarried women as identified in this study, could be logistically challenging due to the confidential nature of this information, could rather lead to stigmatization in society, and is therefore not advisable. Although this study did not focus on HIV-positive women, previous studies have shown the increased HPV incidence and reduced

clearance among HIV-positive women and consequently recommend screening among this group of women (36). This suggests offering regular cervical cancer screening for women attending HIV clinics. The actual impact and feasibility of the risk stratifications discussed above needs to be further studied.

4.3 What influence could the natural history of HPV and cervical cancer have on the screening strategy?

The longitudinal study showed that only 6.7% (7/104) of the women screened had persistent HR-HPV infection and 93.3% cleared their initial HR-HPV infection after a period of 4 years. The persistence and clearance rates seen are concordant with findings from other studies over such a long time period (32). Among the women with persistent infection, one case or CIN2 and one invasive cervical cancer were diagnosed, which is consistent with other studies showing that persistent infection with HR-HPV types leads to increased risk of cervical lesions or even cervical cancer (37). Therefore, women with persistent infection should be managed with higher priority (38).

Incorporating repeat HPV testing and especially genotyping in cervical cancer screening strategies to identify women with persistent infection could be a useful approach to reduce the number of women in need of follow-up. However, repeat testing requires highly organized screening programs that can guarantee the recall of women identified at risk. The high loss to follow-up rate already seen during the cross-sectional study with 48% of HR-HPV positive women after about 12 months post initial screening (31), indicates the logistical challenges for repeat testing in LRS. Consequently, official recommendations by WHO for cervical cancer screening propose as few screening rounds and visits to the health facilities as possible, namely “screen-and-treat” or “screen, diagnose and treat” approaches, for LRS (15). Once organized screening programs are implemented and work successfully, the idea of integrating repeat testing and incorporating persistence results in the overall screening and treatment algorithm may be reconsidered.

4.4 Is HPV-based cervical cancer screening feasible in rural areas of developing countries with the example of Ghana? – Additional aspects

In order to conclude on the question of feasibility of HPV-based cervical cancer screening in Ghana, a number of additional aspects and observations need to be considered. These include challenges discussed in the scientific and political

communities or that arose during the implementation and realization of the ACCESSING study.

4.4.1 Cervical cancer awareness

One important factor is the limited awareness and knowledge about cervical cancer that could highly influence screening uptake. A study conducted in Elmina, Ghana, has shown that the majority of women had never heard about cervical cancer nor about its prevention and treatment options (39). Another study revealed several psychological barriers towards cervical cancer screening in Kumasi, Ghana, such as stigmatization of women with cervical cancer, the lack of spousal support to seek screening services, as well as cultural taboos towards the gender of the healthcare provider (40). The ACCESSING study also assessed the acceptability of self-sampling as well as the awareness regarding cervical cancer. However, these results are not yet published, will constitute my colleagues' doctoral thesis and are therefore not shown as part of this doctoral thesis. It is widely recommended though that sustainable screening programs should include community mobilization by engaging young girls and women but also boys, men, leaders in the communities and key stakeholders to build trust and awareness (15).

4.4.2 Sample collection

The collection of samples during the ACCESSING study went exceptionally well and was characterized by highly motivated CHWs that allowed the recruitment of 2002 women, of which only 20 women (1%) had to be excluded, across the North Tongu District within only 5 weeks (31). This motivation may be due to the special nature of a one-time screening program that had been prepared and announced long prior to screening as well as a small allowance that was paid for the additional work load. Nevertheless, it shows that screening in the local communities with the support of CHWs can reach out to a great number of women that may otherwise not access screening opportunities and consequently increases screening coverage. This in consequence has been shown to improve the population-level health gains as compared to increasing the number of screening events in a lifetime (22).

4.4.3 Human resources for a screening programme

Concerns regarding the additional workload, if such a screening program would be implemented into the routine work, had been expressed by various occupational groups involved, despite the implementation of the ACCESSING study into the existing CHPS system. Bottleneck of particular concern was the laboratory, especially since the HPV genotyping was conducted in Berlin and only sample processing and HPV oncoprotein testing was performed at the Catholic Hospital Battor. The choice of HPV test will greatly depend on the laboratory capacity and vice versa influence its work flow. But also CHWs and nurses expressed their concerns of implementing screening into their routine work. CHWs for example are obliged to rotate between the districts annually. Consequently, continuous training was needed and reliable delegation to new staff coming into the district essential, especially since the algorithm in this study required recall of HR-HPV positive women. Similarly to other projects in LRS, this study was highly driven by the motivation and competencies of the lead gynaecologist at the Catholic Hospital Battor. Depending on the triage and treatment strategy chosen for HR-HPV positive women in a long-term screening program, relying on one person for triage (e.g. colposcopy) or treatment (e.g. LEEP and further surgeries) can create a serious bottleneck.

4.4.4 Costs of HPV-based screening

A major aspect for implementation that could not be assessed as part of this study is the cost-effectiveness of cervical cancer screening. Overall it has been shown in other countries that HPV-based cervical cancer screening is cost-effective. Nevertheless, cost-effectiveness does not equally mean affordability for the respective country or the individuals in need of screening (28). Many governments in LMICs rely on subsidization by other countries governments and the health budget available would not be able to cover the costs for nationwide screening despite its cost-effectiveness. In Ghana, inadequate funding, lack of community awareness and deficient political interest have been identified in the past as factors for limited success of prevention programs (14). Study participants reported a monthly income of less than 25 USD, which will make it hardly possible to afford screening services without governmental support. Yet, emerging initiatives such as the Battor Cervical Cancer Prevention and Training Centre, as a lasting initiative founded after completion of the ACCESSING study (see

www.battorcervicalcentre.org), increase the political pressure and will drive the negotiation regarding affordability of screening approaches forward.

4.4.5 Treatment of screen-positive women

This study mainly focused on the feasibility of screening for HPV infection. A key point not to be forgotten though is the treatment of identified lesions for successful early prevention of invasive cervical cancer. WHO states that the decision for a suitable screening and treatment strategy should be based on factors such as harms and benefits, risk of loss to follow-up, the availability of equipment and human resources needed (15). A study conducted in South Africa showed that immediate treatment of HR-HPV positive women with cryotherapy reduced the incidence of CIN2+ (41). This would lead to overtreatment, due to the fact that not all HR-HPV positive women need treatment. However, modelling results from Uganda suggest that HPV-and-treat approach is still more effective and cost-effective (22). These results should be considered when weighing the options for a suitable cervical cancer screening and treatment algorithm in Ghana.

It is important to note that the here reported practical challenges only refer to the experiences made and concerns expressed during the ACCESSING study in the North Tongu District and do not reflect or are limited to the challenges that could arise in other areas of Ghana or other LRS.

5. Conclusion

WHO Director General Dr. Tedros Adhanom Ghebreyesus has recently called for the elimination of cervical cancer (42), creating an important momentum and motivating governments for the implementation of national cervical cancer screening programs. While the WHO guide for comprehensive cervical cancer control provides clear recommendations for secondary cervical cancer prevention and screening (15), the feasibility of the suggested screening approaches within the individual countries remains unclear and needs to be assessed for each given context. This doctoral thesis assessed the feasibility of an HPV-based cervical cancer screening approach using self-sampling and involving the local CHWs in the recruitment of women to be screened as well as recall of HR-HPV positive women in the North Tongu District, Ghana.

Even though it cannot suggest a universally valid cervical cancer screening and treatment program, based on the results and discussion as presented from the ACCESSING study in Ghana, several lessons can be learned and considerations suggested for the planning of HPV-based cervical cancer screening in rural areas of LMICs.

1) Inclusion of the CHPS system of Ghana with local CHWs in the mobilization, recruitment of women and collection of samples for cervical cancer was very successful and allowed sample collection within a very short time period. Despite the financial incentive provided, including CHW in a screening program proved itself to be highly effective in the North Tongu District and should therefore be continued.
2) The use of self-sampling devices allowed sample collection in the privacy of women's homes without the need to travel to the nearest health facility. The samples collected contained sufficient amounts of DNA for HPV testing and can therefore be recommended, especially in rural and hard to reach settings.
3) The high prevalence of HR-HPV found among the population of the North Tongu District requires triage and potentially follow-up for > 25% of the women screened between the ages 30-49 years. This is a result of the high sensitivity of an HPV-based screening approach and can be a curse and a blessing for large-scale screening approaches, depending on the resources available. It poses the challenge of triage and treatment at a time when researchers and industry are still in the process of developing adequate solutions. At the same time it can be used as

an opportunity to build the health infrastructure and strengthen the health system now, with currently still imperfect triage and treatment options, into which these future innovations can easily be implemented. This is the strategy followed by the Battor Cervical Cancer Prevention and Training Centre, which continuously conducts training for health professionals and implements various methods for detection of HPV and treatment of lesions at various levels of the health system (CHPS with CHWs, nurses, mobile clinics, Battor hospital), to prepare for the innovations to come.

4) Despite the fact that HPV oncoprotein testing seems to be such promising innovation for triage or even highly specific primary HPV testing, it was not feasible to use the self-collected vaginal specimens and achieve reliable test results. Therefore, until this problem can be resolved, alternative triage methods need to be found to detect women at increased risk for cervical lesions and in need of treatment.
5) Using repeat HPV testing to identify women with persistent HR-HPV infection and therefore increased risk of cervical lesions could be a useful way to reduce the number of women that would need to present to the gynaecologist. However, a well-organized system that can guarantee the recall of women after one year is essential for such an approach and may rather be applicable once a screening system has been set up and running successfully for some time.

One of the major strengths of this proof-of-practice study was the diversity of aspects that were addressed (methodological validation, HPV prevalence among the target population, natural history of HPV) and make it possible to provide important recommendations for cervical cancer screening in Ghana and ideas for other LRS. At the same time, it shows that this diversity is needed to decide on a suitable screening approach. There is no “one size fits all” solution but rather a large tool box needed that can be assessed and implemented in each given context.

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Affidavit

I, Amrei Krings certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “Feasibility of HPV-based cervical cancer screening in rural areas of developing countries with the example of the North Tongu District, Ghana.”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

02.10.2019 _____

Date Signature

Declaration of any eventual publications

Amrei Krings had the following share in the following publications:

Publication 1:
Krings A, Dückelmann AM, Moser L, Gollrad J, Wiegerinck M, Schweizer J, Kaufmann AM. Performance of OncoE6 cervical test with collection methods enabling self-sampling. BMC Womens Health. 2018;18(1):68.

Contribution in detail:
Amrei Krings was the lead investigator for the methodological validation underlying this publication. For this she conceptualized and conducted the experiments with supervision of Kaufmann AM. Clinical specimens were collected by Dückelmann AM, Moser L and Gollrad J and tested by Amrei Krings. The results were analysed and validated by Amrei Krings and discussed with Kaufmann AM and Schweizer J. Overall, Amrei Krings was in charge for the project administration. Amrei Krings wrote the original draft for this manuscript with contribution by Kaufmann AM and Schweizer J. All co-authors reviewed and edited the original draft, which was then finalized by Amrei Krings.

Publication 2:
Krings A, Boateng G, Dunyo P, Amuah JE, Adams RA, Adunyame L, Nkansah DO, Wormenor CM, Hansen BT, Gedzah I, Asmah RH, Wiredu EK, Kaufmann AM. Dynamics of genotype-specific HPV clearance and reinfection in rural Ghana may compromise HPV screening approaches. Papillomavirus Res. 2019;7:45-51.

Contribution in detail:
The longitudinal study consisted of two different study parts conducted by separate teams. Amrei Krings was the lead responsible person the methodology of the longitudinal comparison, in detail for data curation by processing samples and HPV genotyping during the second part of the study as well as data analysis, validation and interpretation of the overall study results. For this she was supported by the other co-authors and supervised by Kaufmann AM. Overall, Amrei Krings was in charge for the

project administration. Amrei Krings wrote the original draft of this manuscript and, after revision and editing by the co-authors, finalized the manuscript.

Publication 3:

Krings A, Dunyo P, Pesic A, Tetteh S, Hansen B, Gedzah I, Wormenor CM, Amuah JE, Behnke AL, Höfler D, Pawlita M, Kaufmann AM. Characterization of Human Papillomavirus prevalence and risk factors to guide cervical cancer screening in the North Tongu District, Ghana. PLoS One. 2019;14(6)

Contribution in detail:

The cross-sectional study underlying this publication was carried out in collaboration between two working groups, one at Charité – Universitätsmedizin Berlin and one at Catholic Hospital Battor, Ghana. Amrei Krings supported both working groups by being the lead responsible person for the logistics and training in the laboratory, processing the collected samples in Ghana as well as conducting HPV genotyping and analysing the results in Berlin. Amrei Krings organized the data compilation, curation, analysis, validation and presentation, which was also supervised by Kaufmann AM. Overall, Amrei Krings was in charge for the project administration for the working group at Charité – Universitätsmedizin Berlin and supported the Catholic Hospital Battor in this task. Amrei Krings wrote the original draft of this manuscript and, after revision and editing by the co-authors, finalized the manuscript.

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Signature of the doctoral candidate

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TECHNICAL ADVANCE Open Access

Performance of OncoE6 cervical test with collection methods enabling self-sampling

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Abstract

Background: The paradigm shift from cytological screening to Human Papillomavirus (HPV)-based screening for cervical cancer allows the introduction of new technologies in sample collection and diagnostics. The Onco*E6*™ Cervical Test (OncoE6 Test) is a rapid, easy-to-use lateral flow method detecting HPV16/18 E6 oncoproteins that has proven to detect high-grade cervical lesions with high specificity. If compatible with self-collection samples, this technology might allow for decentralized screening of hard-to-reach populations.

Methods: For technical validation, cervicovaginal lavages were collected from 20 patients with confirmed HPV16+ or HPV18+ invasive cervical cancer. Cervical smears were collected by polyester-tipped swabs and cytobrushes. All samples were applied to the OncoE6 Test and cytobrush samples additionally genotyped.

Results: Lavage, swab, and cytobrush revealed concordant outcome in 18/20 samples. HPV types corresponded with the HPV genotyping by GP5+/6+ PCR analyses. Due to a rare mutation found in the E6 antibody binding site one sample was not detected, another sample had very low cellularity.

Conclusions: Overall, vaginal lavages are technically adequate for the OncoE6 Test. Combining self-sampling with oncoprotein rapid testing to detect women with highest risk for severe dysplasia or cancer may allow for secondary cancer prevention in settings where other screening modalities were unsuccessful to date.

Keywords: Low-resource, Self-collection, Oncoprotein testing, Cervical cancer screening, HPV testing

Background

Despite successes in reduction of cervical cancer related mortality by the introduction of screening programs in developed regions, the worldwide cervical cancer incidence remains high, especially in developing countries. The World Health Organization (WHO) estimates more than 550.000 new cases annually [1]. Cervical cancer is a relatively rare consequence of high-risk human papillomaviruses (hr-HPV) infection and the WHO has classified 12 h-HPV genotypes detectable in > 95% of invasive cervical squamous carcinoma [2, 3]. While hr-HPV infection has a relatively high prevalence of 5–20% in many regions, > 20% can be observed in regions of high HIV prevalence and in women < 25 years old [1]. The HPV types 16 and 18 account for more than 70% of cervical cancers [4].

The association of cervical cancer with hr-HPV (virtually 100% of cervical squamous cell carcinomas are caused by HPV) has motivated the development of molecular screening tests detecting presence of HPV, and WHO recommends the introduction of such screening tools [5]. Generally, HPV tests are characterized by high sensitivity but suffer from low specificity for true disease due to many infections resolving spontaneously or not leading to cervical cancer (HPV infection rarely result in cervical cancer) [6].

A necessary pathogenic event during cervical cancer carcinogenesis is the upregulation of HPV encoded oncoprotein expression. The maintained expression of HPV oncoproteins E6 and E7 is a prerequisite for invasive cervical cancer to develop, while levels of the E6

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and E7 oncoproteins and the corresponding mRNAs are at a very low level in non-transforming HPV infections and low grade dysplasia [7, 8]. These findings motivated the development of the Onco***E6***™ Cervical Test (OncoE6 Test), a technology directly detecting elevated levels of the E6 oncoprotein of HPV types 16 and 18 [9]. The OncoE6 Test relies on genotype-specific mouse monoclonal antibodies (mAbs) to HPV 16 E6 and HPV 18 E6 oncoproteins; these mAbs are used in the format of a lateral flow assay (strip test) of high robustness [9].

The OncoE6 lateral flow assay was validated in numerous clinical studies, and initially only physician collected specimens were used in those studies. For example, in a large (~ 7500 subjects enrolled) screening study conducted in several rural areas in China, the OncoE6 Test had the highest specificity for detection of CIN3 and cervical cancer among all tests involved (compared to *care*HPV and VIA) at 98.9%; the sensitivity of the OncoE6 Test for CIN3+ was 53.5% for all lesions detected and 70.3% when stratified for lesions positive for the HPV types 16/18/45. In this study, the OncoE6 Test had by far the highest positive predictive value (PPV) for CIN3 of 40.8%, while the PPV of HPV testing and VIA was < 10%; the negative predictive value was 99.4% [10].

In low resource settings, tests with high specificity that are also compatible with self-sampling would be advantageous as they facilitate cervical cancer screening of women in remote locations and of women who are hesitant to present to physicians, while reducing unnecessary referrals [11, 12]. We therefore investigated compatibility of the OncoE6 Test with specimens collected by self-sampling. We chose to use cervicovaginal lavages that had previously been shown to be compatible with HPV genotyping [11, 13, 14]; we compared OncoE6 Test performance on self-sampling device collected specimens with physician collected swabs and the cervical brush samples. For this truly technical comparison we sampled from women with histologically confirmed cervical cancers caused by HPV genotypes 16 or 18, and we tested the different sampling methods with regard to the Onco E6 Test, comparing the results to the molecular HPV genotyping performed on the cytobrush samples.

Methods

Patient and samples

Patients were eligible for the study if they presented with HPV 16 or 18 positive invasive cervical cancer, if they have not had a history of conisation or other treatments for cervical cancer, if they were not pregnant and gave their written consent to participate in the study. Ethical approval for this study was granted by Charité Universitätsmedizin Berlin ethical review board (EA1/168/13).

From each patient three different samples were collected in the following order: a vaginal lavage sample (1) taken with the self-sampling device Delphi Screener (Rovers Medical Devices, Oss, Netherlands), a dry sample (2) collected with a sterile polyester tipped swab (#25–806 1PD, Puritan Medical Products) and a cytobrush sample (3) that was applied to PreservCyt storage media (ThinPrep, Hologic Inc., Marlborough, MA, USA). Before the swab and cytobrush sampling, a speculum was inserted and these two samples were collected from the cervix or, if visible, directly by touching the tumour. All three samples were collected by a physician. The samples were stored at − 20 °C until processing.

OncoE6 test

All three samples were applied to the Onco***E6***™ Cervical Test (Arbor Vita Corporation, Fremont, CA, USA) following the manufacturer's instructions. The lavage sample (1) was centrifuged and the whole cell pellet resuspended in lysis buffer. The entire lavage sample was used for the OncoE6 Test to assure highest yield of cells and hence the best possible results. The swab sample (2) , was transferred into a tube for lysis without any further preparation, according to the standard protocol provided for the OncoE6 Test. Of the cytobrush sample (3) in PreservCyt storage media, 5 out of 20 ml of cell suspension were centrifuged and the cell pellet was resuspended in lysis buffer and subsequently applied to the OncoE6 Test. The protocol provided by Arbor Vita Corporation for PreservCyt samples recommends as little as 2 ml cell suspension. We used 5 ml to assure the expected amount of cells would be sufficient.

The OncoE6 Test results for all three sample types were compared to the HPV genotyping outcome, and subsequently the lavage and cytobrush sample results were compared to the swab-based test result.

HPV DNA-based testing

HPV genotyping was performed using the cytobrush sample (3) kept in storage media; 1 ml of this cell suspension was used. DNA was extracted using the QIAmp DNA mini kit (Qiagen, Hilden, Germany) and following the manufacturer's instructions. The extracted DNA was submitted to genotyping using the BSGP5+/6+ PCR approach followed by a Luminex-based readout [15]. The outcome constituted the reference result for HPV genotype status of the patient.

Additional analysis

For all cytobrush samples DNA concentrations were measured using the NanoDrop 2000 (Thermo Scientific, Wilmington, USA) device. It was not possible to measure DNA concentration from the lavage and swab sample, as those were fully used for the OncoE6 test.

One sample (#5) was applied to DNA sequencing of the E6/E7 gene region in the HPV genome (Eurofins

Genomics GmbH, Ebersberg, Germany). Primers encompassed the E6 region of HPV 16 (NCBI accession #: NC_001526) and had the following nucleotide sequences:

Forward: TTGAACCGAAACCGGTTAGT
Reverse: AGATCAGTTGTCTCTGGTTGC

Results

Complete sample sets (a "sample set" consists of: lavage, swab, and cytobrush) were obtained from 20 patients with histologically confirmed invasive cervical cancers; subjects had been pre-selected for infections with HPV types 16 and/or HPV 18; the patients presented at the Clinic for Gynaecology or Radiology, Charité University Hospital in Berlin for treatment. The median patient age was 45 years, ranging from 26 years to 64 years. The patients came to the clinic for surgery and/or radiochemotherapy, at which occasion the samples were collected. None of the patients had previously received treatment for cervical cancer (some patients had to be excluded from the analysis because their medical record revealed prior treatment for cervical cancer).

HPV genotyping results

The genotyping results obtained at the time of sample collection confirmed that 15 out of the 20 cervical cancer patients were positive for HPV 16 and the remaining 5 patients for HPV 18. Three patients each were also positive for additional high-risk HPV types albeit at lower virus load or with a borderline positive result. None was positive for HPV 16 and HPV 18 simultaneously.

OncoE6 test results

The outcome of the OncoE6 Test for lavage, swab and cytobrush samples was highly consistent (see Table 1). E6 oncoprotein positive types by the OncoE6 Test also corresponded to the HPV types 16 or 18 as detected by BSGP5+/6+ PCR genotyping from the cytobrush reference sample. One sample (#14) had a very faint double positive result for HPV 18 and 16 (HPV16 weak) in the lavage sample, and two sample sets showed partially discordant results, warranting further examination as described below.

Verification of discordant assay results

Sample set # 5 (see Table 1) repeatedly tested negative for the OncoE6 Test with all three sample types (Delphi Screener lavage, Swab, and Cytobrush), while the L1-based BSGP5+/6+ PCR MPG HPV typing clearly indicated presence of HPV 16. This sample was therefore examined for mutations in the HPV 16 E6 DNA sequence, hypothesizing that such mutations might alter the E6 oncoprotein binding to the anti-E6 specific monoclonal antibodies (mAb) applied in the OncoE6 Test. The primers chosen encompassed the sequence coding for the epitope recognized by the respective mAb included in the OncoE6 Test. The sequence analysis revealed a single nucleotide exchange (point mutation) at N120 from adenine to guanine, resulting in an altered E6 protein amino acid sequence in position 6 (Gln to Arg). Another point mutation was detected at N350 from thymine to guanine, coding for position 90 of the E6 protein amino acid sequence and resulting in a change from Leu to Val. We hypothesize that the mutation in position 6 reduced binding strength of the epitope to the anti-E6 mAb used in the OncoE6 Test, thus resulting in a false negative outcome.

The second discordant sample set (# 15), tested negative for HPV 16 with the Delphi Screener lavage sample

Table 1 HPV Testing results by sampling method (lavage, swab & cytobrush)

Sample number	DNA Concentration cytobrush sample (ng/μl)	HPV genotyping result from cytobrush (MFI values)		OncoE6 Test result		
				Lavage	Swab	Cytobrush
1	208,9	18 39	(128) (61)	18	18	18
2	30,4	16 33 52	(2020) (54) (231,5)	16	16	16
3	10,3	16	(375)	16	16	16
4	41,3	18	(38)	18 (weak)	18	18
5	34,0	16	(713)	neg	neg	neg
6	68,8	16	(1025,5)	16	16	16
7	27,3	18	(34)	18	18	18
8	63,1	16	(1698)	16	16	16
9	2,8	16	(867)	16 (weak)	16	16
10	33,7	16 33bl[a] 35bl[a] 39bl[a]	(4985) (5) (6) (5)	16	16	16
11	54,8	16 39	(4203) (6)	16	16	16
12	Not available	16	(2799,5)	16	16	16
13	Not available	16	(1008,5)	16	16	16
14	Not available	18 56	(112) (14)	18, 16 (weak)	18	18
15	6,0	16 59bl[a]	(19) (16)	neg	16	neg
16	132,5	16	(1700)	16	16	16
17	71,7	16	(2969,5)	16	16	16
18	6,0	16	(2317)	16	16	16
19	68,7	18 43	(134) (8,5)	18	18	18
20	22,2	16	(2031)	16	16	16

[a] borderline result

and the cytobrush sample, but tested positive for HPV 16 from the swab sample (the latter in concordance with the control genotyping). Possible reasons for these false negative results could be insufficient cellularity in the respective samples. To judge cellularity of the false negative sample we compared DNA content of the cytobrush samples by NanoDrop measurements as an indirect measure of possible cellularity problems. A low DNA concentration was seen in the cytobrush collected sample with only 6 ng/μl while positive samples had a mean content of 51.9 ng/μl (range 2.8 to 208.9). Sample # 9 and #18 had low DNA concentrations in the cytobrush samples as well with 2.8 and 6 ng/μl, respectively, while the OncoE6 results for these samples were positive.

Based on these test results the sensitivity to detect HPV 16 or 18 positive cervical cancers by the OncoE6 Test with the Delphi Screener lavage and the cytobrush sample in PreservCyt media was determined at 90% and for the swab sample at 95%. After exclusion of sample set #5 due to the E6 epitope mutation described above, sensitivity can be adjusted to 95%. For the swab sample, sensitivity was 100% (Table 2). Specificity cannot be calculated, as none HPV 16 or 18 negative patients were included in this initial study enrolment.

Discussion

The shift from cytology-based cervical cancer screening to molecular-based screening opens the path for self-sampling in conjunction with nucleic acid based HPV tests and potentially also with HPV oncoprotein tests [12]. The Delphi Screener is a self-sampling device that is easy to use, well accepted by the users and that results in sufficient material for HPV DNA testing [11, 14, 16]. This device, however, had never been validated in conjunction with the OncoE6 Test, for which most studies up to date were done using swab samples. Here, we performed a technical validation study using the Delphi screener in conjunction with the OncoE6 Test. For this, we selected 20 patients with HPV 16 or HPV 18 positive histology confirmed cervical cancers. Three different sample types were obtained (Delphi lavage, swab, cytobrush) and performance of OncoE6 Test performance was determined for these sample types, using histology as the gold standard for pathology and the L1-based BSGP5+/6+ PCR Multiplexed Genotyping with Luminex readout as the standard for HPV status. The result suggests high sensitivity for detection of cervical cancer by the OncoE6 Test using all three sample types, the self-sampling device generated specimens, the swab samples and also the cytobrush liquid media-based samples.

Table 2 OncoE6 Test Positivity rates by sampling method

Samples tested (n=19[a])	Lavage	Swab	Cytobrush
OncoE6 pos.	18 (95%)	19 (100%)	18 (95%)
OncoE6 neg.	1 (5%)	0 (0%)	1 (5%)

[a] One sample excluded due to E6 epitope mutation

Two of the 20 samples revealed discordant outcome resulting in a sensitivity of 90% for the self-sampling device, however, this may not be applicable with regard to cervical cancer screening by the OncoE6 Test in a population wide setting, as substantiated by the outcome of our further analysis.

One sample set (# 5) was negative in OncoE6 testing for all three sampling methods, despite a strong positive signal for HPV 16 in the L1-based genotyping assay. Sequencing of the E6-specific epitope coding gene segment revealed a non-silent mutation within the E6 gene sequence, suggesting that the resulting epitope has reduced binding strength to the anti E6 mAb used in the OncoE6 Test. Review of the literature for description of E6 specific mutations revealed several studies from various countries (e.g. Congo, Morocco, Romania, China). The mutation we found in our sample set # 5, however, was not described in any of the studies [17–25]. We therefore conclude that this mutation represents a very low frequency mutation and will thus not influence the clinical performance of the OncoE6 Test in the general population, since it is highly unlikely to find this same mutation in relevant numbers elsewhere. Future analyses of discrepant screening by OncoE6 Test results should include interrogation for this mutation.

A major objective of our study was to assess whether or not the Delphi Screener lavage sample self-sampling device is in principle technically compatible with the OncoE6 Test; we therefore focused on samples from HPV 16/18 positive histologically confirmed cervical cancer patients. Excluding the one inadequate sample from our analysis, sensitivity for the self-sampling device among cervical cancer patients selected for this technical validation is at 95% (18/19 samples correctly detected) for the detection of HPV 16 or 18 induced cervical cancers using the Delphi Screener lavage. It can be expected that such very rare mutations do not reduce sensitivity of the OncoE6 test in population-based screening significantly.

The second sample set (# 15) resulting in discrepant outcome showed negative results for the lavage sample as well as the cytobrush sample with the OncoE6 Test, while the result for the swab sample was positive for E6 oncoprotein. It was noticeable that HPV genotyping performed from the cytobrush sample in PreservCyt media showed very low signal strength with the semi-quantitative BSGP5+/6+ PCR with Luminex readout, suggesting the sample was very low on HPV positive cells. To investigate further, we measured the DNA concentration in the cytobrush samples and this sample had a DNA concentration of only 6 ng/μl. Potentially, only few cancer cells were collected, or the tumour was not

directly accessible for sample collection, resulting in negative test results for the cytobrush and the lavage sample. It is possible, therefore, that the number of E6 expressing cells was too low, thus resulting in a negative OncoE6 Test. In contrast, sample #9 and #18 showed a low DNA concentration, suggesting low cellularity of the sample, but in this case it did not seem to have an impact on the genotyping results, which presented positive with high signal strength. As hypothesized previously, self-collected samples bear the risk of not containing enough biological material [26]. Also the location of the lesion, its size, viral load, and smear quality can vary between patients. Data from other studies indicate that even lesions that are too small to be seen in Colposcopy can yield enough E6 protein to be detected in the OncoE6 test [27]. In addition, samples collected with such devices are not specific to the lesion/tumour or the cervix but are cervico-vaginal samples that can contain various types of cells. This might affect the use of such samples in the OncoE6 Test. Since it is difficult to assess how many "correct" cells are collected with self-sampling devices, the impact on sensitivity and specificity of the test needs to be investigated further in population-based screening studies.

Weighing the advantages of wider population benefits due to extended screening possibilities against the drawbacks of a potentially limited sensitivity, we conclude that the Delphi Screener-based lavage sampling represents a very promising approach in conjunction with E6 oncoprotein testing. The undisputed advantages in ease of use and acceptance by the use of self-sampling in conjunction with the high positive predictive value and high specificity of disease detection, as demonstrated for the OncoE6 Test in population-based sampling studies [10], represent important features for effective cervical cancer screening. Elsewhere, the capacity of the OncoE6 Test to stratify risk and to predict >CIN3 at a time point where lesions are not yet visible has been described. In the study of Zhang et al., it was found that women who tested positive for E6 oncoprotein had a ten year cumulative incidence rate of 53.0% for >CIN3 [27]. This could potentially reduce over-referral and allow early and effective treatment of women at high risk, which is of great importance in low-middle income countries (LMICs) and in difficult to access populations.

Our study outcome warrants further investigation in larger population-based studies. The focus of our investigation was foremost to prove the technical feasibility of this approach, and we therefore concentrated on patients with invasive disease positive for HPV 16 or 18. This does not allow any conclusion on the sensitivity and specificity to be found among women with CIN2 or CIN3 lesions. Future studies should include a more typical screening population and thus allow determination of the clinical specificity of self-collection in conjunction with the OncoE6 Test and assess its usability as a screening test for early detection of lesions. The lower specificity of self-sampling devices has already been mentioned by Arbyn et al. [13] and should also be further investigated for this combination of self-sampling device and OncoE6 test.

Using self-sampling devices and E6 oncoprotein testing for cervical cancer screening would allow highly effective selection of women with a high-risk for cervical lesions. Reaching out by self-sampling to women who normally may not benefit from the screening activities or even avoid participation due to cultural barriers could enhance success of preventive strategies [28]. This is especially useful for decentralized cervical cancer screening in resource-constrained settings. A self-collection of lavage samples with the Delphi Screener allows sample collection without a doctor's visit. Once a positive test result is obtained, the patient would be called to be referred to triage and treatment. Such approaches have been shown to motivate patients to consult a gynaecologist [28].

Conclusions

This technical validation shows promising results for E6 oncoprotein testing from lavage samples obtained by a self-collection device, from swab samples and from cytobrush samples, yet further studies are be needed to investigate the feasibility in the general population. The feasibility of self-sampling in conjunction with the highly specific oncoprotein-based test may constitute an important element of cervical cancer secondary prevention in regions and resource constrained settings that could not be served in the past.

Abbreviations

HPV: Human Papillomavirus; Hr-HPV: high-risk human papillomavirus; LMICs: low-middle income countries; mAbs: monoclonal antibodies; OncoE6 test: Onco*E6*™ Cervical Test; PPV: positive predictive value; WHO: World Health Organization

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

AK and AMK designed the study protocol. MW and JS instructed on the usage of the self-sampling device and the OncoE6 Cervical Test usage with the various sample types used. AMD, LM and JG collected samples and evaluated clinical data from women presenting with cervical cancer at the Department of Gynaecology and Radiology, with AK performing the experiments and were therefore all responsible for data acquisition. AK analysed the data and interpreted it together with AMK. AK drafted the first manuscript. All authors gave input or revised the final manuscript critically. All authors read and approved the final manuscript, and take public responsibility for its content.

Ethics approval and consent to participate

Ethical approval for this study was granted by Charité Universitätsmedizin Berlin ethical review board (EA1/168/13) and patients gave their written consent to participate in the study.

Competing interests

AK The authors declare that they have no competing interests.
AMD The authors declare that they have no competing interests.
LM The authors declare that they have no competing interests.
JG The authors declare that they have no competing interests.
MW is founder of Mysample Device Diagnostics B.V. and former of Delphi Bioscience, the company producing the self-sampling device Delphi Screener before acquisition by Rovers Medical Devices.
JS is an employee and shareholder at Arbor Vita Corporation, producing the OncoE6 Cervical Test.
AMK The authors declare that they have no competing interests.

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Dynamics of genotype-specific HPV clearance and reinfection in rural Ghana may compromise HPV screening approaches

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ABSTRACT

Persistent Human Papillomavirus (HPV) infection is a prerequisite for cervical cancer development. Few studies investigated clearance of high-risk HPV in low-and-middle-income countries. Our study investigated HPV clearance and persistence over four years in women from North Tongu District, Ghana.

In 2010/2011, cervical swabs of 500 patients were collected and HPV genotyped (nested multiplex PCR) in Accra, Ghana. In 2014, 104 women who previously tested positive for high-risk HPV and remained untreated were re-tested for HPV. Cytobrush samples were genotyped (GP5 + /6 + PCR & Luminex-MPG readout) in Berlin, Germany. Positively tested patients underwent colposcopy and treatment if indicated.

Of 104 women, who tested high-risk HPV + in 2010/2011, seven (6,7%; 95%CI: 2.7–13.4%) had ≥1 persistent high-risk-infection after ~4 years (mean age 39 years). Ninety-seven (93,3%; 95%CI: 86.6–97.3%) had cleared the original infection, while 22 (21.2%; 95%CI: 13.8–30.3%) had acquired new high-risk infections with other genotypes. Persistent types found were HPV 16, 18, 35, 39, 51, 52, 58, and 68. Among those patients, one case of CIN2 (HPV 68) and one micro-invasive cervical cancer (HPV 16) were detected.

This longitudinal observational data suggest that single HPV screening rounds may lead to over-referral. Including type-specific HPV re-testing or additional triage methods could help reduce follow-up rates.

1. Introduction

Cervical cancer is the fourth most common cancer in women, causing approximately 266.000 registered deaths worldwide every year. In many low-and-middle-income countries (LMICs) it is the second most common cancer in women, representing a major burden with the number of deaths accounting for 85% of the total cervical cancer mortality rate globally [1]. Approximately 99.7% of cervical cancers are caused by persistent infection with HPV genotypes, which are classified as high risk [2]. However, HPV infections and resulting lesions can be transient with, for example, only about 5% of cervical intraepithelial lesion (CIN) grade II lesions progressing to invasion or 22% to carcinoma in situ [3]. It is postulated that probably less than 50% of women with CIN3 develop invasive cervical cancer within 30 years [4]. Nevertheless, it is known that persistent infection with high-risk HPV increase the relative risk of developing high-grade cervical intraepithelial lesions and invasive cancer [5,6]. Interestingly, the prevalence of different high-risk HPV types differs between infection and disease. In Western Africa HPV 16, 58, 18, 35 and 52 are the most prevalent types in normal cytology, while in cervical cancer HPV 16, 18, 45, 59 and 35 are found most often [7]. One study from Ghana shows HPV 18, 59, 45 and 16 as the most prevalent types in descending order in confirmed cervical cancer cases with HPV 18 being present in 47.4% of the cancer tissues [8]. Compared to the worldwide genotype distribution within cervical cancer samples, for which the most common HPV types were 16, 18, 31, 33, 35, 45, 52, and 58 [9], this

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order indicates regional differences. It has also been shown that the carcinogenic potential for high-grade lesions differs between the oncogenic HPV types. While the cumulative risk for the development of CIN2+ after 14 years is 42.8 for HPV 16, it is only 8.1 for HPV 59 [10].

Persistence of different HPV types is an important phenomenon for the development of cervical cancer and its natural history. Elfgren et al. showed in a small group that all women who continuously had genotype-specific high-risk HPV persistence developed CIN2+ lesions within six years [11]. Despite this knowledge still relatively few studies have reported persistence or clearance rates of HPV and the associated risk of developing cervical cancer, especially in LMICs. In a population-based cohort in Costa Rica, for example, a clearance rate of 55% of carcinogenic HPV infections was observed after six months of follow-up [12]. In Scotland, a clearance rate of 51.9% was seen after a period of 14 months [13]. Other studies from Costa Rica, Colombia, and Zimbabwe found clearance rates of 67%, 77% and 73% within 12 months [12,14,15]. These different clearance rates highlight the need to further investigate the natural history of HPV and cervical cancer in various countries and settings.

This knowledge is of fundamental importance, since it greatly determines future screening strategies and algorithms of HPV-based screening for the prevention of cervical cancer, as is currently recommended by WHO [16]. We had an opportunity to compare genotype-specific HPV infections in a cohort of Ghanaian women. Our study presents some rare data on clearance and persistence of HPV infections found in a cohort of 104 women from the North Tongu District of Ghana over the period of four years.

2. Materials and methods

2.1. Initial screening study

The recruitment strategy and patient flow for this study is presented in Fig. 1. As part of a cervical cancer screening project in 2010/2011, 3000 women attending the Battor Catholic Hospital in the North Tongu District, Volta Region in Ghana, were screened for cervical cancer and its precursor lesions. Women older than 15 years, with no history of cervical cancer and not pregnant were recruited into the study. Women who were unable to undergo speculum vaginal examination, including virgins, those who had undergone hysterectomy or conisation and women who were unable to give consent were excluded. Smear samples were collected using an Aylesbury spatula and sent to the Cytopathology Laboratory of the School of Allied Health Sciences, University of Ghana for cytological examination. Five hundred (500) of these 3000 women were randomly selected for HPV genotyping using nested multiplex PCR according to Sotlar et al. [17] at the Laboratory as part of a PhD project undertaken by GB and supervised by EKW. The following genotypes were tested for: 6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66, and 68. Ethical clearance for this study was provided by the University of Ghana School of Allied Health Sciences Ethics and Protocol Review Committee (Ref. No. SAHS ET/AA/24A/2010). Signed/thumb-printed written/translated informed consent was obtained from all women participating in the screening.

All the cytology reports were released to the Battor Hospital for management and follow up. However, the HPV testing was started in 2011 and completed in 2013.

2.2. ACCESSING study – Follow-up

In 2013 collaboration between the Catholic Hospital Battor, Ghana and the Charité University Hospital Berlin, Germany started with the topic "Adequate Cervical cancer Capacity Building, Education and Screening with new Scientific Instruments in Ghana", namely the ACCESSING study. This study was independent from the previous study within a new collaboration but concentrating on the prevalence of HPV and cervical cancer in the same geographical area. Ethical clearance for this study was given by the Ghana Health Service Ethical Review Committee (Ref. No. GHS-ERC: 05/05/13) in October 2013.

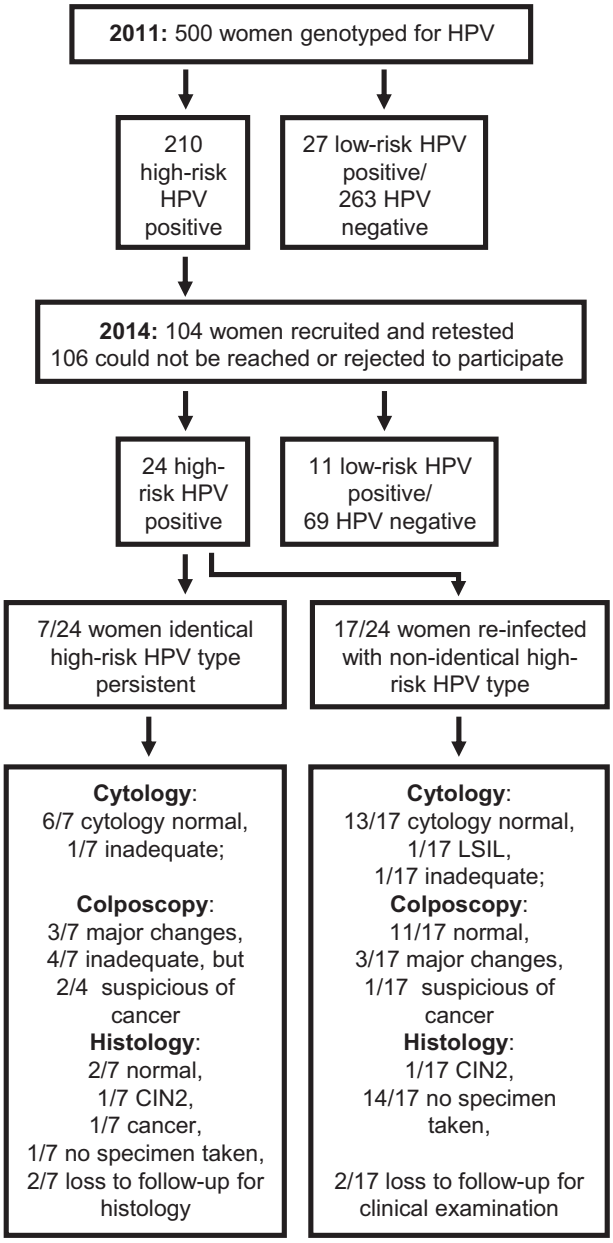

Fig. 1. Flow chart of HPV screening studies in 2011 and 2014 presenting inclusion of 104 women by HPV test outcome and clinical diagnosis for persistence analysis. Abbreviations: LSIL – low-grade squamous intraepithelial lesion; CIN2 – cervical intraepithelial lesion grade 2.

Recruitment for an initial pilot study started in March 2014 in order to validate sample collection, logistics and diagnostic testing. For this pilot study 150 women between the ages of 18–65 years with a previous history of HPV infection or abnormal cytological result but currently not pregnant were recruited at the gynecological clinic of the Catholic Hospital Battor. Women who had remained untreated since 2011 and fulfilled the criteria of this study were re-called and asked to participate again for final follow-up as part of this pilot. The participants were recruited on a convenience approach. Potential differences in age and HPV type positivity between women agreeing to and declining recruitment in 2014 were assessed using chi-square test of independence. Samples were collected for cytology and HPV genotype testing. Cytological examination was done at the Department of Pathology, University of Cape Coast, Ghana. HPV genotyping was done on

cytobrush samples by BSGP5+/6+ PCR followed by Luminex-MPG read-out detecting the genotypes 6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 54, 56, 57, 58, 59, 66, 68a, 68b, 70, 72, 73, 82, and 90 at Charité Universitätsmedizin Berlin, Germany [18]. Differences in age between women with cleared or persistent high-risk HPV infection as well as newly infected women were tested using chi-square test. Those who tested positive for high-risk HPV were recalled for colposcopy and if indicated by colposcopy LEEP was performed. Based on the histology results from the LEEP specimen additional treatment was provided if needed. Every woman screened in 2014 filled out a questionnaire asking for general demographic data (e.g. age, education, and income level per month) as well as specific risk factors such as age at first intercourse, number of sexual partners, etc.

For reasons of consistency the HPV high-risk classification of WHO was used, classifying the HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 as carcinogenic HPV genotypes. Additionally, we consider HPV 66 and 68 as high-risk. The remaining types are defined as potentially carcinogenic or low-risk types [19]. It is important to note that all carcinogenic high-risk types are detected with both assays, however some low-risk types are not detected consistently with both assays. The nested PCR according to Sotlar et al., as performed in Accra, Ghana, included HPV 44, which was not tested with the BSGP5+/6+ PCR according to Schmitt et al. in Berlin, Germany, while HPV types 26, 53, 54, 57, 70, 72, 73, 82, and 90 were not tested by the nested PCR method used in Accra. In the following analysis clearance and persistence of low-risk types will therefore only refer to the HPV types 6, 11, 42, and 43 included in both assays.

3. Results

3.1. Characteristics of study participants

Attempt was made to contact all the women who tested high-risk HPV positive in the Accra study in 2010/2011 and re-call them but only 104 out of the initial 210 women could be reached and agreed to participate in the ACCESSING pilot study in 2014, and were re-screened for HPV.

Comparing the age and HPV type positivity between the women who agreed to participate and those who were not re-screened, we observed only small differences. The median age of women re-screened was 34 years in 2010/2011 and of those not re-screened 35 years. This difference was not significant (chi-square test; p-value = 0.336). When tested to see if the initial positivity for the individual 14 high-risk HPV types in 2010/2011 differed between the two groups, we found that less women re-screened were initially HPV52 positive compared to those not re-screened (chi-square test; p-value = 0.001) and more of those re-screened were initially positive for HPV68 (chi-square test; p-value = 0.028).

Among the women re-screened in 2014 almost one third (30.8%) of the women had no formal education, 21.2% completed elementary school, 47.1% completed secondary school and 1% continued with post-secondary school education at the time of re-screening. More than half (64.4%) of the women screened were married and the majority of women (73.1%) had two to three sexual partners, as shown in Table 1. The results from the questionnaire showed that more than two thirds of the women (68.3%) did not use contraceptives.

3.2. Results initial screening study

Among the 104 high-risk HPV-positive women in the 2010/2011 study, 56 had single infections and 48 multiple infections and the mean number of high-risk HPV types was 1.6. The most prevalent high-risk HPV types were HPV 18 (26.0%), 58 (23.1%), 52 (22.1%), 68 (20.1%), and 66 (14.4%). HPV 16 was detected in one sample (1.0%), the only squamous cell carcinoma detected. HPV 43 was the most prevalent low-risk type with 19.2%.

Table 1
Sociodemographic and behavioral characteristics of women included in HPV persistence analysis at time point of inclusion in 2014 (n = 104).

		n	%
Age			
	Mean 39.39 yrs		
	20–30	27	25.96
	31–40	35	33.65
	41–50	22	21.15
	51–60	16	15.38
	60 +	4	3.85
Education			
	None	32	30.77
	Primary	22	21.15
	Junior High School	35	33.65
	Secondary	14	13.46
	Post Secondary	1	0.96
Income level per month			
	< 100 GH¢	65	62.50
	100–250 GH¢	16	15.38
	251–500 GH¢	4	3.85
	> 500 GH¢	2	1.92
	missing data	17	16.35
Occupation			
	Farmer/Trader	70	67.31
	Food Vendor	4	3.85
	Hairdressing	5	4.81
	Nurse	2	1.92
	Seamstress	6	5.77
	Unemployed	2	1.92
	Other (Baker, Caterer, Student, etc.)	10	9.62
	missing data	5	4.81
Marital status			
	Single	6	5.77
	Have a steady partner	9	8.65
	Living with someone (unmarried)	6	5.77
	Married	67	64.42
	Divorced	7	6.73
	Widowed	9	8.65
# of sexual partners			
	1	19	18.27
	2–3	76	73.07
	> 3	9	8.65
# of children			
	None	9	8.65
	1–2	25	24.04
	3–4	36	34.62
	5–6	16	15.38
	> 6	17	16.35
	missing data	1	0.96
Age at first intercourse			
	< 15	3	2.88
	15–18	45	43.27
	19–22	36	34.62
	> 22	8	7.69
	N/A	12	11.54
Contraceptive use			
	None	71	68.27
	Abstinence	11	10.58
	Injectable	13	12.50
	Norplant/Jadelle	1	0.96
	Pill	8	7.69
Current smoking			
	Yes	3	2.88
	No	101	97.12

Abbreviations: GH¢ - Ghana Cedi (local currency); N/A - no answer.

3.3. Results ACCESSING study

In 2014 no high-risk HPV infection was proven in 76.9% (80/104) after approximately four years of follow up, with 73.1% (76/104) being completely HPV negative while 3.8% (4/104) were positive for low-risk HPV (6, 11, 42, or 43) only. Twenty-four (23.1%) women were high-risk HPV positive. Altogether, 45 high-risk HPV infections and five low-risk infections were found. Of the 24 high-risk positive women, 13 had

Table 2
Prevalence in 2011 and 2014 and Persistence/Clearance/Reinfection of high-risk HPV by type among study participants (n = 104).

HPV Type	Prevalence of high-risk HPV in 2011		Prevalence of high-risk HPV in 2014		Persistence		Clearance		Reinfection
	n	%	n	%	n	%	n	%	n
HPV 16	1	1.0	9	8.7	1	100.0	0	0.0	8
HPV 18	27	26.0	0	0.0	0	0.0	27	100.0	0
HPV 31	7	6.7	2	1.9	0	0.0	7	100.0	2
HPV 33	7	6.7	0	0.0	0	0.0	7	100.0	0
HPV 35	11	10.6	2	1.9	1	9.1	10	90.9	1
HPV 39	6	5.8	5	4.8	1	16.7	5	83.3	4
HPV 45	1	1.0	4	3.8	0	0.0	1	100.0	4
HPV 51	13	12.5	4	3.8	1	7.7	12	92.3	3
HPV 52	23	22.1	9	8.7	3	13.0	20	87.0	6
HPV 56	8	7.7	1	1.0	0	0.0	8	100.0	1
HPV 58	24	23.1	1	1.0	0	0.0	24	100.0	1
HPV 59	1	1.0	3	2.9	0	0.0	1	100.0	3
HPV 66	15	14.4	2	1.9	0	0.0	15	100.0	2
HPV 68	21	20.2	3	2.9	1	4.8	20	95.2	2
All HPV	165		45		8/165 (95% CI: 2.1–9.3%)	4.8	157/165 (95% CI: 90.7–97.9%)	95.2	37
In 104 women	104		24		7/104 (95% CI: 2.7–13.4%)	6.7	97/104 (95% CI: 86.6–97.3%)	93.3	21.2% (22/104) (95% CI: 13.8–30.3%)

Abbreviation: CI - Confidence interval.

single and 11 multiple infections with a mean number of 1.9 high-risk HPV types (Range: 1–5). The most prevalent high-risk HPV genotypes found were HPV 16 and 52 (8.7% each), 39 (4.8%) and 45 and 51 (3.8% each) among the 104 women. HPV 18 was diagnosed in none of the samples. HPV 70 was the most prevalent low-risk type found in 4.8% (5/104) of the patients. This low-risk type had not been tested for in the 2011 study. HPV 43 was found in only one of the patients.

3.4. Longitudinal comparison

Comparing the HPV genotyping results from 2010/2011 with the results from 2014, 6.7% (7/104; 95% CI: 2.7–13.4%) women had ≥1 persistent high-risk HPV type (Table 2). None of the low-risk HPV types persisted. Persistent HPV types found were HPV 16, 35, 39, 51, 52 and 68. HPV 52 persisted in three women, while the other HPV types were found persistent in only one woman, respectively. One woman had two persistent HPV types (HPV 51 and 52). Based on the comparison of type-specific infections, the clearance rate found within four years was 93.3% (97/104; 95% CI: 86.6–97.3%) and rate of reinfection with a new high-risk HPV type was 21.2% (22/104; 95% CI: 13.8–30.3%).

Clearance rates of HPV remained high throughout all age groups. Proportions of women with reinfection with other high-risk HPV types was 8.0% and 20.0% in the age groups 20–29 and 30–39 years, while it was more than double in relative terms (30.8% and 33.3%) in the age groups 40–49 and 50–59 years (Table 3). Most persistent cases could be seen in the younger age groups (20–29 years with 2/7 cases and 30–39 years with 3/7 cases) compared to the remaining two cases across the older age groups (40–49 years with 1/7 cases and 50–59 years with 1/7 cases).

When testing the association between persistence, clearance, and reinfection rates across the different age groups with chi-square test no significant association was seen.

Table 3
Persistence, Clearance, and Reinfection between 2011 and 2014 by age group (n = 104).

Age group		Persistence		Clearance		Reinfection	
	n	n	%	n	%	n	%
20–29	25	2	8.0	23	92.0	2	8.0
30–39	30	3	10.0	27	90.0	6	20.0
40–49	26	1	3.8	25	96.2	8	30.8
50–59	15	1	6.7	14	93.3	5	33.3
60+	6	0	0.0	6	100.0	1	16.7
Unknown	2	0	0.0	2	100.0	0	0.0
Total	104	7	6.7	97	93.3	22	21.2
95% CI			2.7–13.4%		86.6–97.3%		13.8–30.3%
chi-square test			1.03		1.03		4.67
p-value			0.795		0.795		0.197

Abbreviation: CI - Confidence interval.

3.5. Clinical outcome

Women testing positive for high-risk HPV in 2014 underwent colposcopic examination and a smear was taken for cytology. If a lesion was suspected, women underwent LEEP for further histological confirmation and decision on further treatment was based on these results. Out of the seven women who were positive for the same HPV type as in 2011, six women had normal cytology results. One cytology was unsatisfactory and the woman was recalled for a repeat smear but was lost to follow up. Her colposcopic examination had been inadequate due to the lack of visibility of the transformation zone, with no visible changes. She had persistent HPV 52 infection.

One of the six women with normal cytology had normal colposcopy results, major changes were seen in three women, and two women were suspected to have cervical cancer. Due to these abnormal findings during colposcopy, four women underwent LEEP surgery, which showed no lesion to be present in two women but revealed one case each of CIN2 and microinvasive cervical cancer (Fig. 1). One of the two women with suspected cervical cancer from colposcopy did not return to the clinic for histological confirmation and potential treatment. She had persistent HPV 51 and 52 infections. The case of CIN2 was a woman with persistent infection with HPV 68 and the case of microinvasive cancer was caused by persistent infection with HPV 16. The other two women having HPV 35 or 52 persistent HPV infections had no lesions. Unfortunately, two of these seven women were lost to follow up despite several attempts to convince them for triage and treatment.

Out of the 17 women who were re-infected with high-risk HPV genotypes 13 had normal cytology, one woman was diagnosed with LSIL, for one woman the Pap-smear was unsatisfactory and two women were lost to follow-up without cytology results. On colposcopic examination of the 15 triaged women 11 women were diagnosed as normal, three women showed major colposcopic changes, of whom one was cytologically confirmed LSIL, and one woman was suspicious for cancer. The woman suspicious of cancer had normal cytology results

and a LEEP biopsy specimen revealed a CIN2 lesion on histological examination. No further histological confirmation was obtained for any of the other women.

4. Discussion

This comparative analysis of two independent HPV genotyping projects in smears from the same group of women sampled years apart was used to determine the persistence and frequency of resolution of prevalent HPV infections.

The most remarkable result emerging from this study is that after an exceptionally long follow-up period of four years, high-risk HPV infection persisted in only 6.7% (95% CI: 2.7–13.4%) of the women. Almost three quarters of the women had completely cleared their high-risk HPV infection and were HPV-negative at the time of follow up, without receiving any form of treatment in the intervening period. It is important to note that a total of 21.2% (95% CI: 13.8–30.3%) had been re-infected with new high-risk HPV types. Such infections become only apparent as new and non-persistent by genotyping HPV assays and could have been mistaken as persistent high-risk HPV infections. Persistent infections would be classified with a higher risk of malignant transformation compared to those with a type change or clearance [11].

The clearance and persistence rates found are concordant with other studies having such a long follow-up period. In a study from Columbia among women with a median age of 29 years a clearance rate of 93% was found after five years of follow up [14]. Even at shorter follow-up periods remarkably high clearance rates can be found. At six months for example clearance of 55% and at 12 months of 67% is reported for oncogenic HPV in a study population in Costa Rica [12]. In South Korea 77% of high-risk HPV cleared within 18 months [20]. The median time to clearance reported by Giuliano et al. is 9.8 months for oncogenic types and 4.3 month for non-oncogenic types [21]. Muñoz et al. report a similar trend in which oncogenic types have longer time to clearance compared to non-oncogenic types and especially HPV 16 infections clear after significantly longer time intervals compared to low-risk HPV and other high-risk non α-9 types [6].

There was no evidence for an association between clearance or persistence rates and age. This could be due to the low number of women who were diagnosed with the same HPV type after four years. There was also no association between reinfection and the different age groups. Several studies have shown though that women at younger age and after initiation of sexual activity tend to have high prevalence rates but that infections clear quickly, in contrast to women at older ages, which tend to have lower prevalence rates, resulting rather from persistent infections [22].

With the switch of many national cervical cancer screening programs to HPV-testing based screening these results may support the available evidence on clearance that is needed to decide on the duration between screening visits.

WHO recommends to initiate screening programs based on HPV testing instead of cytology in countries that have no program in place [16].

Interestingly, none of the low-risk HPV types detected in 2011 persisted. This is also consistent with findings from other studies. In Columbia, low-risk HPV types were found to have a lower persistence rate compared to HPV 16 [14]. In a study from Zimbabwe persistence of low-risk HPV types was 21.9% over a median period of 21 months, which was significantly lower compared to high-risk HPV types with 37.3% persistence [15].

Different oncogenic HPV types have different duration until clearance and different carcinogenic potential. Within our study population we found the HPV types 16, 35, 39, 51, 52 and 68 to be persistent. Among these HPV 16 and 68 caused confirmed invasive cervical cancer and CIN2 within the follow-up period, respectively. The woman found with persistent HPV 16 infection was diagnosed with invasive cervical cancer after an abnormal colposcopic finding and LEEP biopsy. It was remarkable that she presented with a normal cytological result of NILM "Negative for Intraepithelial Lesion and Malignancy" in 2014. The histologically examined LEEP specimen indicated micro-invasive cervical cancer, upon which a radical hysterectomy was performed.

In comparison, among the 17 women who were found newly infected with high-risk HPV during the second testing round in 2014 only one case of CIN2 was detected. Cytology detected one woman with LSIL, from whom no biopsy could be taken.

While HPV 16 clearly is the main driver of cervical cancer and can be found in most cases of cervical cancer worldwide [9], HPV 68 is not very common in high-grade lesions [23]. Only in South Africa has HPV 68 been found to be among the "Top 10″ cervical cancer-causing HPV types [7].

HPV 16 has been reported in the literature as the HPV type that persists significantly longer or in other words has lower clearance rates compared to other HPV types. Bulkman et al. showed in a population-based cervical screening cohort with 44.102 women that the type-specific clearance rates of high-risk HPV differ with HPV 16 and HPV 31 having significantly lower clearance rates in women with normal cytology results at baseline [24]. Moreover, HPV 16 is also the type with the highest detection rate (12%) of CIN3+ within 18 months after normal cytology at baseline [24].

This has also been presented in other studies. Wheeler et al. for example showed that the 2-year cumulative risk of developing CIN3+ for women with equivocal or mild cervical cytological abnormalities is different depending on the HPV type present. For HPV 16 the risk was 39.1%, while for the other high-risk types the cumulative risk was only 7.9% [25]. This trend can also be seen at 10 years in a study conducted by Khan et al. There, the cumulative incidence rate observed for CIN3+ among women with normal cytology at baseline was 20.7% for HPV 16+, and 17.7% for HPV 18+ women. In contrast, it was 1.5% for women who were non-HPV 16/18 high-risk positive [26]. A recent study conducted by Smelov et al. reported on the cumulative risk for CIN3+ from a 14-year follow-up study. The risk when HPV 16-positive at baseline was 34.5 as compared to 20.7 when positive for any high-risk type or 0.9 when HPV-negative [10]. Additionally, the analysis mentioned above by Elfgren et al. showed that all 40 women followed with HPV genotyping and persistent identical HPV type infection developed CIN2+ lesions within six years of persistence irrespective of the type the women remained positive for [11].

Based on the knowledge gained about type-specific clearance rates and risks for the development of CIN3+, repeat genotype testing within the regular screening algorithm for cervical cancer prevention should be considered and could greatly influence follow-up of HPV positive women [27]. This is of particular importance, considering that cytology-based screening algorithms have been shown to be inadequate in many settings for reasons such as limited sensitivity, limited human and financial resources, and poorly developed healthcare services and access to primary healthcare facilities [28]. Therefore, HPV testing is recommended for cervical cancer screening in low-resource settings by WHO since 2013 [16]. It is important though to consider the high sensitivity of HPV testing and the low positive predictive value for detection of cervical cancer of the HPV tests currently available for screening. It helps in finding more CIN2+ cases, compared to cytology, that are in need for treatment but at the same time may lead to a very high referral rate of HPV positive patients for follow-up and highlights the need for further triage strategies of HPV positive patients [29,30]. High referral rates will result in a burden for the already low-staffed health care systems in LMICs. While persistence of high-risk HPV with the identical type according to Elfgren et al. should be recognized as a leading risk factor for the development of cervical lesions and if possible included in the screening algorithms [11], they also highlight the difficulties in managing follow-up for persistence patients.

Although the risk of developing lesions from persistent HPV infections is increased, we found five patients who had not developed any lesions, even after the extended time of persistent infection of four

years. Castle et al. have described this phenomenon in a group of patients from Guanacaste, Costa Rica, who had persistent high-risk HPV infection without developing lesions, even after a mean follow-up period of 6.5 years. He suggests that an unmeasured susceptibility factor may hinder women from clearing their HPV infections but not necessarily result in the development of cervical lesions [31].

Bearing in mind the fact that in 2014 23% of the women were high-risk HPV positive, yet direct comparison of the HPV types revealed persistent infection in only 6.7% of the women, the importance of HPV genotyping tests is highlighted compared to group tests for high-risk HPV types. Especially when using persistent HPV infection as the basis for clinical follow-up, additional 16.3% (17/104) of the women would have been classified high-risk HPV persistently positive with a generic group test while they actually had cleared the original HPV types and were re-infected with a new high-risk type. Khan et al. suggested at least HPV 16 and 18 genotyping to increase the positive predictive value of HPV testing and potentially even create screening algorithms that immediately refer HPV 16/18 positive patients for colposcopy [26]. This has also been evaluated in the ATHENA study and confirmed the effectiveness of direct referral of HPV 16 or 18 positive women with NILM cytology for colposcopy as it is currently recommended in the 2006 American Society of Colposcopy and Cervical Pathology guidelines [32]. Genotyping was also shown to provide high sensitivity and specificity for the possible recurrence of disease after CIN treatment, as a 'test of cure' [27,33]. Yet most of the currently available results are focused on partial genotyping only. Therefore, larger prospective cohort studies investigating the type-specific clearance, especially of non-HPV 16/18 types, such as HPV 31, 33 and 45 are needed.

Within our study we did not analyse potential risk factors for persistence of infection due to the small study group. However, in the literature, aspects such as age, cigarette smoking, parity, and oral contraceptive usage are described as characteristics associated with persistence of HPV infection [14] and could be included into risk assessment for HPV genotype persistence. We did not find an association between persistence and age, most likely due to the small study group with persistent infection, as mentioned above.

Despite the fact that this study reveals some important insight into the long-term persistence of high-risk HPV infected women with a time period of four years before follow-up, there are limitations due to the opportunistic character of this analysis. The study follow-up and longitudinal analysis was not intended from the beginning. The greatest limitation is that HPV genotyping was performed with two different HPV genotyping tests in 2011 and 2014. This may lead to misclassification of persistent infections due to the different targets of the tests (E6 and E7 vs L1) as well as the potentially different sensitivities and specificities and thus the results need to be interpreted with caution. HPV 18 was seen very frequently during the screening round in 2010/2011, yet no case of HPV 18 was seen in 2014 anymore. Beside from the clearance of HPV 18, this could be due to different sensitivities of the tests for HPV 18. Furthermore, the genotyping tests were also performed in different laboratories (Accra, Ghana and Berlin, Germany) resulting further in potential inter-laboratory differences, which limits the reliable comparison of HPV types found and, therefore, the classification of persistent infection.

HPV testing was performed only at two time points within the follow-up period despite the recommendations proposed by Muñoz and Koshiol [5,6]. Especially Muñoz suggested that only incident infections lasting longer than the median duration of infection should be considered persistent. A common problem is though that this recommendation is not yet widely used. As a consequence, this study cannot provide additional information on the time to clearance except from this point prevalence.

In addition, the quality of cytology performed on the samples from 2014 may not be perfectly adequate. At least one confirmed case of invasive cervical cancer was classified with normal cytological findings and two additional histologically confirmed cases of CIN2 were missed. Furthermore, cytology and colposcopy was only performed on women tested high-risk HPV positive in 2014 and not on those women tested HPV negative.

5. Conclusion

The main finding that only 6.7% of high-risk HPV types persisted after four years is of significant importance and should be considered for future prospective studies evaluating potential screening algorithms. Especially at a time of paradigm shift from cytology to HPV testing for the prevention of cervical cancer, further research on the significance and feasibility of genotyping for screening is urgently needed. This longitudinal observational data suggest that single HPV screening rounds may lead to over-referral. Including type-specific HPV re-testing or additional triage methods could help reduce follow-up rates. Despite the limitations mentioned but also due to the highly interesting and important results seen in this pilot trial the performance of further prospective long-term cohort studies investigating clearance and persistence of individual HPV genotypes especially in high prevalence populations in LMIC is warranted.

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Authors' contribution

All authors gave input or revised the final manuscript critically. All authors read and approved the final manuscript and take public responsibility for its content.

Additional contribution by authors:
Conceptualization: GB, RHA, EKW, AMK
Data curation: AK, GB, PD
Formal analysis: AK, GB, JEA, EKW, AMK
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Methodology: AK, EKW, JEA, RHA, AMK
Project administration: AK, GB
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Declarations of interest

None of the authors have any conflict of interest to declare.

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PLOS ONE

RESEARCH ARTICLE

Characterization of Human Papillomavirus prevalence and risk factors to guide cervical cancer screening in the North Tongu District, Ghana

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Abstract

Introduction

This population-based study aimed to fill the knowledge gap on Human Papillomavirus (HPV) prevalence and associated sociodemographic risk factors of the general population in the North Tongu District, Ghana. These results are needed to guide cervical cancer prevention efforts, as the leading type of female cancers.

Methods

A cross-sectional study including 2002 women in the North Tongu District, Ghana investigated HPV prevalence and associated sociodemographic risk factors. Women were recruited by geographical distribution through the local community-based health system and samples collected using a self-sampling device. For HPV genotyping BSGP5+/6+-PCR with Luminex-MPG readout was used. Multivariate logistic regression analyzed sociodemographic risk factors for HPV positivity.

Results

Of 2002 self-collected samples, 1943 were eligible, contained sufficient DNA and provided valid HPV genotyping results. Prevalence of single high risk HPV types was 32.3% and of multiple high risk types 9.7%. The five most common detected HPV types were HPV16 (7.4%; 95%CI: 6.3–8.7), HPV52 (7.2%; 95%CI: 6.1–8.5), HPV35 (4.8%; 95%CI: 3.9–5.8), HPV59 (4.7%; 95%CI: 3.8–5.8), HPV56 (3.9%; 95%CI: 3.1–4.8). Highest prevalence was observed among women aged 18–24 years, while age 25–54 years was inversely associated with high risk HPV positivity in multivariate analysis. Sociodemographic risk factors

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identified were i) having any sexual partner, ii) more partners increased the odds for high risk HPV positivity, iii) independently from this marital status, in particular not being married.

Discussion & conclusion

Most importantly, the high risk HPV prevalence detected from this study is higher than estimates reported for Western Africa. This needs be considered, when deciding on the cervical cancer screening algorithms introduced on a wider scale. Follow-up and triage, depending on the methods chosen, can easily overburden the health system. Self-sampling worked well and provided adequate samples for HPV-based screening. Women with increasing number of sexual partners and not being married were found to have higher odds of being high risk HPV positive, therefore could be a higher prioritized screening target group.

Introduction

Global situation HPV & cervical cancer

Cervical cancer is the 4th most common cancer among women in the world leading to about 528.000 registered new cancer cases and 266.000 cervical cancer deaths annually [1]. About 99% of cervical cancer cases are caused by persistent infection with Human Papillomaviruses (HPV) [2] and genotypes HPV16 and HPV18 cause approx. 70% of the global cervical cancer cases [3]. Regional differences exist for the HPV genotype distribution in cervical cancer. In Sub-Saharan Africa HPV16, 18, 45, 35 and 33 were identified as the most common types [4], while globally HPV16, 18, 45, 33 and 31 are most common in the respective order [3]. With different risks for and time to cancer progression as well as possible protection from HPV vaccination, genotype prevalence and distribution are important factors to investigate in each world region.

Low-middle income countries (LMIC) carry the greatest global burden of cervical cancer with about 85% of incident cases and 87% of annual deaths occurring there [1]. Reasons for this are late presentation at the health facilities [5], poorly developed health systems, lack of financial and technical resources as well as human capacity to diagnose and treat cervical cancer, and often also lack of awareness [6]. This high rate of cervical cancer incidence is projected to increase by 90% until 2030 when considering the current increase in incidence as well as aging and population growth [7]. Factors such as the increasing number of HIV-positive patients further contribute to this projection [7]. While HPV vaccines are available and provide effective primary prevention, their accessibility in LMICs is still limited mostly to initiatives by the Global Alliance for Vaccines and Immunization (GAVI). Furthermore, the available vaccines do not cover all high risk HPV types and therefore the need for simple, affordable and acceptable secondary prevention remains inevitable. A review of policy documents and interviews with key stakeholders from Tanzania, Kenya and Uganda shows that although the advantage of HPV testing for cervical cancer screening is understood, screening remains rare and is often offered to women outside the recommended age range. Programs are underfunded, resulting in low coverage and insufficient quality [8].

In Ghana cervical cancer is the 2nd most common female cancer among women at the age of 15 to 44 years and the 2nd leading cause of cancer deaths [9]. With a crude incidence rate of 18.6 per 100.000 annually, cervical cancer is even more common in Ghana compared to the

Western Africa region with 16.8 per 100.000 [1, 9]. At the Korle Bu Teaching Hospital Accra 57.8% of the women presenting with gynecological cancer had cervical cancer [10].

Despite this high burden of disease little is known about the prevalence of HPV infection and its risk factors in the general population of Ghana. Studies investigating this are very rare and limited to small studies ($n<230$) or specialized populations (e.g. referral population, cervical cancer patients, pregnant women). Prevalence rates stated are for example 10.7% among 75 women attending the gynecology outpatient clinic in Accra [11], or 42% and 76.6% among 100 HIV negative and 107 HIV positive women from Kumasi, respectively [12]. A study among pregnant women from the Western Region of Ghana detected 13.9% of the women to be high risk HPV positive [13]. Few studies focused on the HPV genotype distribution among cervical cancer patients. The Pathology Department at the University of Ghana found the most common HPV types among 230 cervical cancer patients to be HPV18 (47.4%), HPV59 (42.2%), HPV45 (37.4%) and HPV16 (9.0%) with 52.2% having multiple HPV types [14]. Thus, no large population-based study depicting the HPV prevalence has been published from Ghana to date.

The ACCESSING study

ACCESSING is an acronym for "*A*dequate *C*ervical cancer *C*apacity building, *E*ducation and *S*creening by new *S*cientific *IN*struments in *G*hana" and a program funded by the Deutsche Gesellschaft für Internationale Zusammenarbeit (GIZ) and the German Rotary Voluntary Doctors (GRVD). The program was conducted during a hospital partnership in collaboration between the Catholic Hospital Battor in the Volta Region, Ghana, and the Charité—Universitätsmedizin Berlin, Clinic for Gynecology, Germany. The aims of this program were to assess the HPV and STI prevalence with a cross-sectional study design in the general population, to evaluate a potential screening algorithm for cervical cancer screening on its feasibility, and to build the capacity to independently and autonomously introduce cervical cancer screening. The program was conducted from October 2013 until February 2017 including two pilot studies with 250 and 150 women screened at the Catholic Hospital Battor, respectively, and a main study with 2002 women screened from the rural and urban communities in the North Tongu District.

This manuscript presents the results from the ACCESSING main study investigating HPV prevalence, genotype distribution, and the sociodemographic risk factors associated with high risk HPV positivity. This shall help decision makers to identify adequate cervical cancer screening methods and potentially prioritized risk groups for screening.

Methods

Study population

This evaluation of the ACCESSING program is a cross-sectional study conducted in the North Tongu District, Ghana in a collaboration of the Catholic Hospital Battor, Ghana and the Charité—Universitätsmedizin Berlin, Germany. Women in the age of 18 to 65 years without a history of cervical cancer and who were not pregnant were recruited.

Sample size calculation was based on a HPV prevalence estimate of 21.3% and the objective to identify a clinically relevant difference of 2.5% to this suggested prevalence [15]. Adjusting for a potential loss to follow-up, loss of sample material or unusable samples of between 5–6% and at a 5% significance level with 80% statistical power, the sample size needed was 2003 [16]. Thus a sample size of 2000 women was proposed to be sufficient to detect a clinically important difference.

Ethical clearance for this study was given by the Ghana Health Service Ethical Review Committee (Ref. No. GHD-ERC: 05/05/13) in October 2013. Signed/thumb-printed written/translated informed consent was obtained from all women participating in the screening.

Sample collection

The number of women included in each village within the North Tongu District was defined based on the population size reported during the latest Population Census from 2010 and adjusted to a sample size of 2000 study participants [17]. Recruitment of study participants was conducted by Community Health Workers (CHW), as part of the Ghana Health Service Community-based Health Planning and Services (CHPS), who are based in the respective villages, on a door-to-door and first-come-first-serve basis.

The samples were collected using an approved (Ghana FDA) self-sampling device, namely the Evalyn brush (Rovers Medical Devices, Oss, The Netherlands). Evalyn Brush samples were self-collected or collected with assistance from a CHW according to manufacturer's instructions and stored dry and at ambient temperature for a maximum time period of 7 days until arrival in the laboratory of the Catholic Hospital Battor, Ghana. Every woman screened filled out a questionnaire asking for general demographic data (e.g. age, education, and income level per month) as well as specific risk factors such as age at first intercourse, number of sexual partners, etc. (see supporting information S1 File for the questionnaire).

Sample processing & HPV genotyping

The brush head was removed from the self-sampling applicator into a 2 ml Eppendorf tube and 1 ml of PreservCyt solution was added and vortexed vigorously for about 1 min. After incubation overnight at room temperature brushes were vortexed again and then removed. 100 μl from this cell suspension were aliquoted and stored at -20˚C for DNA extraction at the Laboratory for Gynecologic Tumor Immunology, Clinic for Gynecology, Charité Universitätsmedizin Berlin.

For DNA extraction, the Maxwell 16 LEV Blood DNA Kit (Promega GmbH, Mannheim, Germany) was used according to manufacturer's instructions. DNA was eluted into 60 μl of elution buffer and stored at -20˚C until further used.

Genotyping of mucosal HPV was done by BSGP5+/6+ PCR followed by Luminex-MPG read-out according to Schmitt et al. 2008 [18] detecting the genotypes 6, 11, 16, 18, 26, 31, 33, 35, 39, 42, 43, 45, 51, 52, 53, 54, 56, 57, 58, 59, 66, 68a, 68b, 70, 72, 73, 82 and 90 at Charité—Universitätsmedizin Berlin, Germany. Clinical performance of this test has been validated and considered useful for HPV-based cervical cancer screening [19]. Results were considered valid if sufficient DNA was present, indicated by a Luminex measured Median Fluorescent Intensity (MFI) of 200 or more for ß-globin, which was used as a proxy for cellular material. The MFI is interpreted qualitatively, indicating if a sample is positive or negative for the respective HPV type, but not used for quantitative information on the viral load of samples.

According to WHO monograph classification HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59 were considered as high risk HPV genotypes. HPV66 and 68a and 68b were defined as probable, HPV26, 53, 73, and 82 as potential high risk and the HPV types 6, 11, 42, 43, 54, 57, 70, 72, 90 as low risk types [20].

Clinical follow-up

Women who tested positive for high risk HPV were recalled to the clinic for cytology and upon abnormal cytology result called again for colposcopy. Based on the diagnosis by the treating gynecologist treatment, such as loop electrosurgical excision procedure (LEEP) or

hysterectomy, was provided. Cytological examination was done at the Department of Pathology, University of Cape Coast, Ghana. HPV testing as well as any clinical follow-up required were provided free of charge for the study participants.

Statistical analysis

Statistical analysis of categorical data was done using high risk HPV positivity as the dependent variable. Univariate and multivariate logistical regression analysis was used to identify potential sociodemographic risk factors for high risk HPV positivity. Variables for multivariate logistic regression were chosen based on their association resulting from univariate analysis as well as from sociodemographic risk factors reported in the literature. The multivariate model was built using forward stepwise selection and the likelihood ratio test to compare the fit of the models. The unadjusted odds ratio (OR), adjusted odds ratio (AOR), 95% confidence intervals (95%CI) and p-values from univariate and multivariate logistic regression were reported as a measure of association. All reported p-values were 2-sided with a significance level of 0.05. For statistical analysis STATA version 15 (StataCorp LLC, College Station, Texas, USA) was used.

Results

From a total of 2002 women samples with a self-sampling brush were collected through the CHW system in the North Tongu District within 5 weeks. Two samples did not reach the laboratory, 18 were excluded from this analysis because they were not within the age range of 18–65 years and an additional 39 samples were excluded due to insufficient DNA present in the sample for HPV testing. Hence a total of 1943 samples were included in the HPV and risk factor analysis (Fig 1). Among the 2002 samples tested this represents sufficient DNA for 98% of samples.

Sociodemographic description

The distribution of sociodemographic characteristics is summarized in Table 1 with a few details described here: The average age of the women tested was 32 years of age. The majority of women completed Junior High School or higher (59.8%) and about half of the women had a monthly income of less than 100 Ghana Cedi (GHS), which calculated to approx. US $ 25 per month (in August 2015). The most common occupations were trading and farming and about 9.5% were students or doing an apprenticeship. Many women recruited were married (42.2%) and had 1–2 children (38.0%), ranging to up to 13 children. The majority did not use any contraceptive (66.0%) and had 2–3 sexual partners. Almost half (48.0%) of the women were between 15–18 years when they had their first sexual intercourse.

HPV prevalence results from the North Tongu District, Ghana

Among the 1943 samples left for analysis, high risk HPV was detected in 32.3% (95%CI: 30.2–34.5) and 9.7% (95%CI: 8.4–11.1) were positive for multiple high risk HPV types. On average women with multiple high risk types had 1.4 high risk types detected (range: 1–7 high risk types). 18.4% (95%CI: 17.7–20.2) were positive for low risk HPV as single or multiple infection and 53.5% (95%CI: 51.3–55.8) HPV negative. Among women aged 30–49 years, the WHO recommended screening age, high risk HPV prevalence was 27.3% (95%CI: 24.3–30.5). Women high risk HPV positive were invited for clinical follow up free of charge. This was structured in cytology and based on indication colposcopy, LEEP and potentially hysterectomy. Results of clinical follow up are presented in the supporting information S1 Fig.

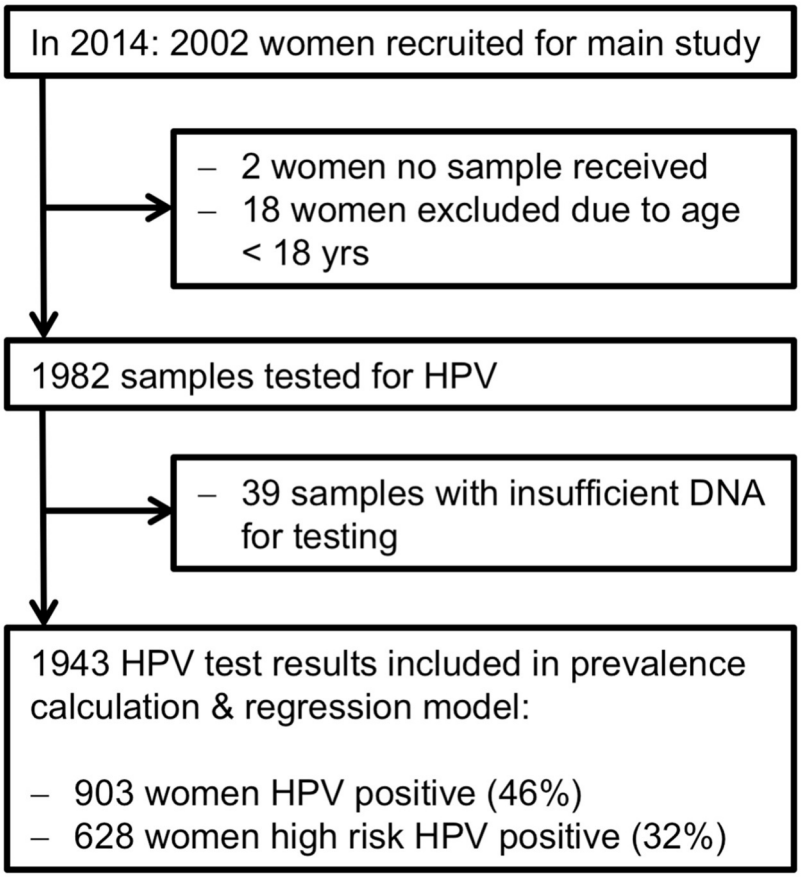

Fig 1. Flow chart of ACCESSING main HPV screening study in 2014.

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The five most prevalent high risk HPV types were HPV16 (7.4%; 95% CI: 6.3–8.7), HPV52 (7.2%; 95% CI: 6.1–8.5), HPV35 (4.8%; 95%CI: 3.9–5.8), HPV59 (4.7% 95% CI: 3.8–5.8) and HPV56 (3.9%; 95% CI: 3.1–4.8) (Table 2). HPV66, as a probable high risk type was detected in 4.3% (95% CI: 3.5–5.3) of the women. The most common low risk type detected was HPV42 (7.1%; 95% CI: 6.0–8.3).

The age-specific HPV prevalence is presented in Fig 2 and was highest among women younger than 25 years (41.7%; 95% CI: 37.5–45.9). With increasing age, prevalence decreases up to 54 years and then increases again in the oldest age group of 55–65 years. Lowest prevalence is seen in the age group 45–54 years with 25.1% (95% CI: 19.3–31.7).

Risk factors associated with high risk HPV positivity

Univariate analysis suggests high evidence for decreasing odds ratio (OR) of high risk HPV positivity for women with the following sociodemographic factors (Table 3). Women at older age had lower ORs for high risk HPV infection compared to those of younger age. Furthermore, women who work as farmers (OR: 0.4; 95%CI: 0.3–0.6; p-value: <0.001), teacher (OR: 0.5, 95%CI: 0.3–0.8; p-value: 0.010), traders (OR: 0.6; 95%CI: 0.4–0.8; p-value: 0.001) or in the health care sector (OR: 0.4; 95%CI: 0.2–0.9; p-value: 0.032) were associated with a decreased OR for infection compared to students or women doing an apprenticeship. The OR increased for women who completed primary (OR: 1.5; 95%CI: 1.1–2.0; p-value: 0.016) or secondary school (OR: 1.7; 95%CI: 1.2–2.5; p-value: 0.002) in comparison to those without any

Table 1. Sociodemographic and behavioral characteristics of eligible study participants (n = 1982).

Sociodemographic characteristics	n	Prevalence in %
Age		
Mean: 31.9 years		
18–24	552	27.9
25–34	744	37.5
35–44	403	20.3
45–54	205	10.3
55–65	78	3.9
Education		
None	346	17.5
Primary	436	22.0
Junior High School	793	40.0
Secondary	254	12.8
Post Secondary	73	3.7
Post Graduate	65	3.3
missing data	15	0.8
Income level per month		
<100 GH¢	989	49.9
100–250 GH¢	329	16.6
251–500 GH¢	106	5.4
>500 GH¢	109	5.5
missing data	449	22.7
Occupation		
Apprentice/Student	188	9.5
Farmer	494	24.9
Hairdresser	82	4.1
Worker in Health care sector	34	1.7
Seamstress	112	5.7
Teacher	74	3.7
Trader	668	33.7
Unemployed/House wife/Retired	129	6.5
Food vendor	46	2.3
Office Employee	30	1.5
Cleaner	9	0.5
missing data	116	5.9
Marital Status		
Single	178	9.0
Have a steady partner	395	19.9
Living with someone (unmarried)	428	21.6
Married	836	42.2
Divorced	86	4.3
Widowed	58	2.9
missing data	1	0.1
Number of sexual partners		
None	23	1.2
1	760	38.4
2–3	964	48.6
>3	210	10.6

(*Continued*)

Table 1. (Continued)

Sociodemographic characteristics	n	Prevalence in %
missing data	25	1.3
Number of children		
None	355	17.9
1–2	754	38.0
3–4	483	24.4
5–6	247	12.5
>6	138	7.0
missing data	5	0.3
Age at first intercourse		
<15	66	3.3
15–18	951	48.0
19–22	628	31.7
23–26	103	5.2
>26	24	1.2
missing data	210	10.6
Contraceptive Use		
None	1308	66.0
Injectable	347	17.5
Pill	140	7.1
Norplant/Jadelle	82	4.1
Condom	65	3.3
Abstinence	24	1.2
IUCD	4	0.2
Other	8	0.4
missing data	4	0.2
Current smoking		
Yes	19	1.0
No	1953	98.5
missing data	10	0.5

Abbreviations: GH¢—Ghana Cedi (100 GH¢ ~ 25US$, exchange rate in August 2015); IUCD—Intrauterine contraceptive device

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educational degree. No such association was seen for women who completed Junior High School. High evidence for increasing ORs of high risk HPV positivity was seen among women who are single (OR: 2.6; 95%CI: 1.8–3.6; p-value: <0.001), or are not married—including those who have a steady partner (OR: 2.6; 95%CI: 2.0–3.4; p-value: <0.001), live with someone (OR: 1.5, 95%CI: 1.2–1.9; p-value: 0.003) or are divorced but not widowed (OR: 1.8; 95%CI: 1.1–2.9; p-value: 0.014), compared to married women. The number of children a woman had steadily decreased her OR of being high risk HPV positive. Other factors such as age at first sexual intercourse, the use of contraceptives and income level did not show high evidence for an association with HPV positivity in univariate analysis.

After multivariate logistical regression only the factors age, marital status and number of sexual partners appeared to have a high association for infection (Table 4). Here, as mentioned above younger age had a higher OR for infection. Being single (AOR: 2.6; 95%CI: 1.8–3.8; p-value: <0.001), having a steady partner but not living together (AOR: 2.2; 95%CI: 1.6–2.9; p-value: <0.001) and being divorced (AOR: 1.9; 95%CI: 1.2–3.0; p-value: 0.011) were highly

Table 2. Prevalence of HPV by genotype among eligible women (n = 1943).

HPV Type positivity	Infections positive (n)	(%)	95% CI
High risk HPV			
16	144	7.4	6.3–8.7
18	72	3.8	2.9–4.6
31	47	2.4	1.8–3.2
33	15	0.8	0.4–1.3
35	93	4.8	3.9–5.8
39	40	2.1	1.5–2.8
45	63	3.2	2.5–4.1
51	58	3.0	2.3–3.8
52	140	7.2	6.1–8.5
56	75	3.9	3.1–4.8
58	45	2.3	1.7–3.1
59	92	4.7	3.8–5.8
Probable high risk HPV			
66	84	4.3	3.5–5.3
68a	28	1.4	1.0–2.1
68b	47	2.4	1.8–3.2
Potential high risk HPV			
26	7	0.4	0.1–0.7
53	72	3.7	2.9–4.6
73	24	1.2	0.8–1.8
82	59	3.0	2.3–3.9
Low risk HPV			
6	51	2.6	2.0–3.4
11	12	0.6	0.3–1.1
42	137	7.1	6.0–8.3
43	23	1.2	0.8–1.8
54	50	2.6	1.9–3.4
57	1	0.1	<0.1–0.3
70	45	2.3	1.7–3.1
72	12	0.6	0.3–1.1
90	87	4.5	3.6–5.5
HPV positivity	**women positive (n)**	**(%)**	**95% CI**
HPV negative	1040	53.5	51.3–55.8
High risk HPV+	628	32.3	30.2–34.5
Single high risk HPV+	440	22.6	20.8–24.6
Multiple high risk HPV+	188	9.7	8.4–11.1
High risk AND probable HPV+	697	35.9	33.7–38.1
High risk AND probable AND potential HPV+	750	38.6	36.4–40.8
Low risk HPV+	405	18.4	17.7–20.2

Abbreviations: OR—odds ratio; CI—Confidence interval; HPV—Human Papillomavirus

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associated with high risk HPV positivity. Living with someone but not being married (AOR: 1.3; 95%CI: 1.0–1.7; p-value: 0.047) was also associated with infection. The more sexual partners a woman had the higher the ORs for infections were (e.g. >3 partners AOR: 5.0; 95%CI: 1.7–14.6; p-value: 0.004). Interestingly, there was already a high association for infection with the first sexual partner (AOR: 3.3; 95%CI: 1.1–9.3; p-value: 0.027).

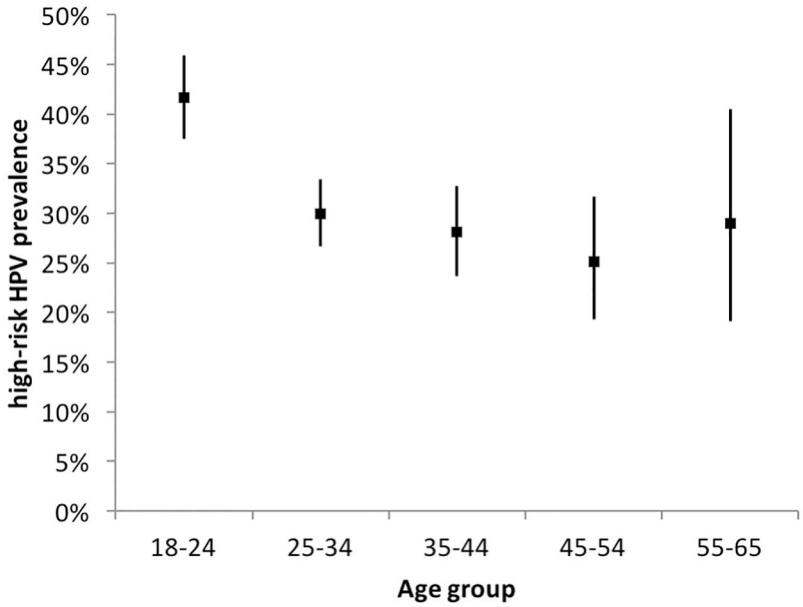

Fig 2. High-risk HPV prevalence by age group.

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Discussion

Sample collection via self-sampling worked exceptionally well in this study. The integration of recruitment and sampling in the responsibilities of CHW system allowed simultaneous screening across the District within only 5 weeks. With 98% of the samples providing valid HPV genotyping results, this sampling method should be considered for large-scale screening programs, especially for widespread communities.

HPV prevalence in Ghana

The results of this cross-sectional HPV prevalence study show an exceptionally high prevalence of the high risk HPV genotypes of 32.3%. This is a 52% increase compared to the initially expected prevalence of 21.3%, which had been used as an estimate for Western Africa in the sample size calculation [15]. Even among women aged 30–49 years, the WHO recommended screening age, high risk HPV prevalence is high and above the estimate for Western Africa with 27.3%.

Looking at some of the other scarce studies conducted among the general population in Ghana, a prevalence of 10.7% was found among 75 women (mean age: 33.3 years) attending the gynecology outpatient clinic in Accra [11], or of 42% among 100 HIV negative women (mean age: 40.9 years) attending the outpatient department of a referral hospital in Kumasi [12]. Schulze et al. reported a prevalence of 13.9% for high risk HPV among pregnant women in Eikwe, Southwest Ghana [13]. Although not directly reflecting the situation in Ghana, the extensive meta-analysis including 1 million women across 5 continents estimates the prevalence for Western Africa at 19.6% after adjusting for various factors such as geographical subregion, mean age of women, HPV testing method etc. [21]. A study from the neighboring country Burkina Faso reports a prevalence similar to our findings of 34.4% among women aged 35.5 years recruited among visitors of an urban medical center [22]. One could hypothesize that women referred to and recruited from a medical center possibly with characteristic symptoms have a higher risk of being HPV positive, may rather be considered a referral

Table 3. Univariate logistic regression analysis of potential risk factors for high risk HPV positivity.

Characteristic	Total	High risk HPV+	OR	95% CI	p-value
Age					**<0.001**
18–24	545	227	Ref.		
25–34	727	218	0.6	0.5–0.8	**<0.001**
35–44	392	110	0.6	0.4–0.7	**<0.001**
45–54	203	51	0.5	0.3–0.7	**<0.001**
55–65	76	22	0.6	0.3–1.0	**0.036**
Education					
None	335	91	Ref.		
Primary	427	151	1.5	1.1–2.0	**0.016**
Junior High School	784	248	1.2	0.9–1.7	0.137
Secondary	248	97	1.7	1.2–2.5	**0.002**
Post Secondary	72	24	1.3	0.8–2.3	0.293
Post Graduate	62	15	0.9	0.5–1.6	0.627
Income level per month					**0.019**
<100 GH¢	976	317	Ref.		
100–250 GH¢	321	87	0.8	0.6–1.0	0.072
251–500 GH¢	100	23	0.6	0.4–1.0	0.054
>500 GH¢	106	28	0.8	0.5–1.2	0.204
Occupation					
Apprentice/Student	183	80	Ref.		
Farmer	487	125	0.4	0.3–0.6	**<0.001**
Hairdressing	82	29	0.7	0.4–1.2	0.203
Health care	34	8	0.4	0.2–0.9	**0.032**
Seamstress	110	44	0.9	0.5–1.4	0.533
Teacher	73	19	0.5	0.3–0.8	**0.010**
Trader	654	201	0.6	0.4–0.8	**0.001**
Unemployed/House wife/Retired	127	47	0.8	0.5–1.2	0.238
Food vendor	43	15	0.7	0.3–1.4	0.293
Office	29	10	0.7	0.3–1.5	0.352
Cleaner	9	2	0.4	0.1–1.8	0.220
Marital Status					
Single	174	77	2.6	1.8–3.6	**<0.001**
Have a steady partner	387	174	2.6	2.0–3.4	**<0.001**
Living with someone (unmarried)	415	132	1.5	1.2–1.9	**0.003**
Married	825	196	Ref.		
Divorced	83	30	1.8	1.1–2.9	**0.014**
Widowed	58	19	1.6	0.9–2.8	0.125
Number of sexual partners					**0.003**
None	23	5	Ref.		
1	750	224	1.5	0.6–4.2	0.404
2–3	938	306	1.7	0.6–4.7	0.276
>3	208	85	2.5	0.9–7.0	0.082
Number of children					**<0.001**
None	342	148	Ref.		
1–2	745	250	0.7	0.5–0.9	**0.002**
3–4	476	138	0.5	0.4–0.7	**<0.001**
5–6	240	61	0.5	0.3–0.6	**<0.001**
>6	135	29	0.4	0.2–0.6	**<0.001**

(*Continued*)

Table 3. (Continued)

Characteristic	Total	High risk HPV+	OR	95% CI	p-value
Age at first intercourse					**0.045**
<15	64	26	Ref.		
15–18	935	319	0.8	0.5–1.3	0.291
19–22	608	188	0.7	0.4–1.1	0.115
23–26	103	31	0.6	0.3–1.2	0.164
>26	24	6	0.5	0.2–1.4	0.179
Contraceptive Use					
None	1278	397	Ref.		
Injectable	341	123	1.3	1.0–1.6	0.079
Pill	138	45	1.1	0.7–1.6	0.710
Norplant/Jadelle	81	33	1.5	1.0–2.4	0.071
Condom	65	19	0.9	0.5–1.6	0.755
Abstinence	24	7	0.9	0.4–2.2	0.842
IUCD	4	0			
Other	8	3	1.3	0.3–5.6	0.696
Current smoking					
Yes	17	5	0.9	0.3–2.5	0.797
No	1917	620	Ref.		
Usage of herbal vaginal preparation					
Yes	246	74	0.9	0.7–1.2	0.422
No	1691	552	Ref.		

Abbreviations: OR—odds ratio; CI—Confidence interval; HPV—Human Papillomavirus; GHC—Ghana Cedi; IUCD—Intrauterine Contraceptive Device

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Table 4. Risk factors associated with high risk HPV positivity from multivariate analysis.

	High risk HPV positivity			Multiple high risk HPV type positivity		
Characteristic	**Adjusted OR**	**95% CI**	**p-value**	**Adjusted OR**	**95% CI**	**p-value**
Age						
18–24	Ref.			Ref.		
25–34	0.7	0.5–0.8	**0.001**	0.7	0.5–1.0	0.083
35–44	0.7	0.5–0.9	**0.013**	0.4	0.2–0.7	**0.001**
45–54	0.6	0.4–0.8	**0.005**	0.7	0.4–1.3	0.243
55–65	0.7	0.4–1.2	0.172	0.6	0.3–1.6	0.331
Marital Status						
Single	2.6	1.8–3.8	**<0.001**	2.5	1.4–4.4	**0.002**
Have a steady partner	2.2	1.6–2.9	**<0.001**	2.5	1.6–3.9	**<0.001**
Living with someone (unmarried)	1.3	1.0–1.7	**0.047**	1.8	1.1–2.8	**0.015**
Married	Ref.			Ref.		
Divorced	1.9	1.2–3.0	**0.011**	3.5	1.8–7.0	**<0.001**
Widowed	1.6	0.9–2.9	0.135	1.4	0.5–4.1	0.579
Number of sexual partners						
None	Ref.			-		
1	3.3	1.1–9.3	**0.027**	Ref.		
2–3	3.7	1.3–10.4	**0.016**	1.1	0.8–1.5	0.675
>3	5.0	1.7–14.6	**0.004**	1.7	1.1–2.8	**0.028**

Abbreviations: OR—odds ratio; CI—Confidence interval

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population and that this reported prevalence is therefore an overestimation. However, our findings support such high prevalence also to be seen among the general population. This is further supported by a different study conducted in Western Burkina Faso finding a prevalence of 38.3% [23] and a prevalence of 33.2% reported from Benin [24]. Other studies report prevalence or type distribution among selected populations, such as women with cervical lesions or even cervical cancer or HIV-positive women, which cannot be compared to this prevalence among the general population. Ghana has an overall low HIV rate of 1.5% and even though the Volta Region with 2.7% has the highest reported HIV prevalence within the country, it is still low compared to other Sub-Saharan African countries and may not explain the high prevalence found [25]. While the prevalence seen here may not be representative for the prevalence in Western Africa or even other regions in Ghana, given the large sample size it is a valid representation of the prevalence in the North Tongu District of Ghana, which is higher than so far reported and expected.

Nevertheless, the majority of women (65.4%) recruited for this study was at the age of 18–34 years with a prevalence of 35.0% for this age group. Looking at the age distribution of the population living in North Tongu district from the 2010 Population and Housing Census, the age group 20–34 years comprises about 51.0% among the 20–64 years old women (similar to the age range meeting the study inclusion criteria) of the North Tongu population and is hence overrepresented among the women recruited for this study. On the other hand the older age group (45–65 years; 34.6% of the study participants) comprises 48.9% among 20–64 years old in North Tongu women's population and is therefore underrepresented here. Since the peak of HPV infection is expected among women aged $\leq$25 years [26], this could cause an overestimation of the actual prevalence among the general population in the North Tongu District.

Additionally, it has been described that although the accuracy of HPV testing from self-collected samples compared to physician-obtained samples is similar, there is a tendency of more HPV types being detected in self-collected samples from the cervicovaginal compartment as compared to physician-collected cervical-targeted sampling [27–30]. This could also lead to an overestimation of the prevalence described here, compared to commonly reported cervical HPV prevalence. Another methodological factor that could influence the reported prevalence of various studies is the sensitivity of testing methods for HPV detection. Different HPV detection methods have slightly different sensitivities and would therefore over- or underestimate the true HPV prevalence in the population.

HPV type distribution

The five most prevalent high risk HPV types found in this study are 16, 52, 35, 59 and 56, in descending order, known to contribute to a total of 59.4% of cervical cancer cases in the African Region, as defined by WHO [31]. The types 35, 56 and 59 are not included in any HPV vaccine, but in this study contribute to a prevalence of 12.4% (some women are positive for two of the mentioned types). Looking at the prevalence of these types in invasive cervical cancer, as reported for Western Africa, all three types are among the top 10 cervical cancer causing HPV types, being prevalent in 16.4% of cancers, with HPV59 alone contributing almost 10% [31]. The prevalence of these non-vaccine covered HPV types shows that certain HPV vaccines may not be as effective in this region as compared to other regions of the world. The prevalence of distinct HPV genotypes varies between world regions and also in the African continent. Reasons could be e.g. the local prevalence of HIV that leads to more multiple and persistent infections by other genotypes than HPV16. Data on prevalence from sufficiently large studies are sparse for Western Africa and a direct comparison cannot be drawn due to

use of different HPV genotyping tests. However, HPV35 (rank 4–5) and 39 (rank 10) have shown a dominating proportion in other African regions [31].

Multiple HPV infections

9.7% of the women in this investigated population had multiple high risk HPV infections. Multiple high risk HPV infections are often reported among HIV positive persons [32–34]. However, it may not fully explain the results seen from the population recruited for this study. As mentioned above, Ghana has an overall low HIV rate, which therefore may not be the only contributor to the high prevalence of multiple high risk infections [25]. Another aspect described to be associated with high prevalence of multiple infections is high promiscuity among sex workers [35]. Among the women recruited 10.6% had more than 3 sexual partners and the mean number of partners is 2.1. The number of sexual partners was associated with single high risk infection, but also with multiple high risk HPV infection from multivariable regression analysis of this study (Table 4). Multiple type positivity is an important factor to investigate, as it increases somebodies risk of infection with additional HPV types and is under debate to also reduce their risk to clear prevalent types, meaning prevalent types persist longer [36]. It also reduces the survival of cervical cancer patients and has a higher rate of distant tumor recurrences [37].

Risk factors for HPV positivity

Based on the self-reported answers provided to the questionnaires several risk factors have been investigated and shown to be associated with high risk HPV infection. Knowledge of these risk factors is of great importance to target women at highest risk of infection with screening programs, especially in countries with limited resources for screening. Three risk factors remained associated with high risk HPV infection after multivariable regression analysis: Age, relationship status, and number of sexual partners.

Transmission of genital HPV and hence infection occurs with the onset of sexual activity and HPV infection has been described to peak among younger women aged $\leq$25 years [26]. This is supported by the results from this study with the highest HPV prevalence of 41.7% found among women younger than 25 years. The multivariate analysis further confirmed this, showing the lowest ORs among older women compared to women younger than 25 years.

Being single or divorced was highly associated with infection compared to being married. Surprisingly, having a steady partner and living with someone but not being married was also associated with infection, even after adjusting for age and the number of sexual partners. This might be due to ambivalent interpretation of these two categories by the study participants. The questionnaires were filled out under supervision of the CHW and that might have influenced the answers given. Possibly different interpretation of relationship length influenced the category of "having a steady partner". Other studies reported that being in a relationship for 12 months or longer is protective for infection, indicating that the duration of a relationship seems to be an important factor [38]. In the literature early age at marriage is also described to be associated with HPV infection [39], which is a factor that was not asked for. Beside from this, the number of sexual partners in a lifetime is one of the most important factors associated with HPV positivity. Various studies could show, similarly to our findings, that an increasing number of sexual partners also increase the OR of being high risk HPV positive [39–42]. While other studies mostly focus on the number of sexual partners as a risk factor and rarely investigate the relationship status, interestingly we see both factors to be independent risk factors. Our results show an association between relationship status and the number of sexual partners, but when including both in the multivariable regression analysis, both remain

independently highly associated with HPV infection. This means that both are independent risk factors despite their association. This has not yet been investigated in other studies.

Other studies found lower levels of formal education to be associated with infection [43]. In our population we can only partially confirm this, since we found primary and secondary education to be associated with higher risk of infection, however we did not see a difference in association between no or post-graduate education. Educational level was also not associated anymore with high risk infection in the multivariate analysis after adjustment. Also, cigarette smoking has been reported to be associated with HPV infection [41]. Since only about 1% of our study participants indicated to smoke we could not assess this risk factor.

Limitations

While this cross-sectional study is sufficiently powered to detect a difference in HPV prevalence compared to the estimate stated for Western Africa, it was not meant to be powered to assess risk factors. The sociodemographic and behavioral characteristics are based on self-reported questionnaires and may therefore be subject to biased answers. The associations we found have to be investigated in larger studies and a similar context.

Additionally, problems with sample genotyping resulted in longer waiting times than expected for the women participating. Since women may travel across the country with the harvest season and also the CHWs responsible for the respective area change locations for training, to mention only a few potential factors, only about half of the positively tested women travelled to the clinic for follow-up. The unexpectedly high prevalence made it difficult for all women to receive colposcopy and therefore the additional cytology triage step was introduced. Therefore, we cannot make any conclusions on the clinical status of all women tested positive and hence excluded the clinical outcome from any further analysis.

Conclusion

The main finding of this study is a higher prevalence of high risk HPV than expected until now. Secondly, we found that three HPV types, namely HPV35, 56 and 59, are among the top 5 types found but not covered by any HPV vaccine even though they are found in 16.4% of cervical cancers in Western Africa [31]. Thirdly, risk factors found for HPV positivity were young age, the number of sexual partners and the relationship status.

Despite the high HPV prevalence found in the investigated population there is currently no national screening program for cervical cancer screening in Ghana. Although the recent national control program for non-communicable diseases (NCDs) states an instituted cervical cancer screening system as a national policy priority, little of the suggested means have been implemented until today [44] and the cervical cancer screening coverage remains low with 2.8% of women aged 25–64 years [45]. This is further highlighted by an analysis including cervical cancer patients from the Catholic Hospital Battor in Ghana, showing that late presentation at the clinic was the main risk factor for cervical cancer diagnosis [5]. The high prevalence of non-vaccine preventable HPV types shows the importance of secondary prevention efforts, even if primary prevention with the available vaccines would be fully implemented. The results of this study can therefore help in tailoring a screening system that is adapted to a higher than expected HPV prevalence.

Self-sampling worked well and provided adequate samples for HPV-based screening. However, the high HPV prevalence found would require further triage and follow-up for a large number of women, which can easily overburden the health system. This is a major aspect when deciding on the feasible cervical cancer screening algorithm introduced on a wider scale.

Supporting information

S1 File. Questionnaire.
(PDF)

S1 Fig. Flow chart of clinical follow up.
(TIF)

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Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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Appendix

Questionnaire used during the ACCESSING study:

1. Hospital card no.	
2. Study ID no. (Use Stickers)	
3. Initials of patient (Surname first)	__ __ __
4. Date of attendance	_____/_____/_____ (*DD/MM/YY*)
5. District	
6. Village	

A. Socio-demographic characteristic

1. Age of patient	__ __ yrs
2. Do you normally reside in this district? (**1**-Yes, **2**-No) 2b. If "No" please specify current place of residence _____	__
3. Highest level of education completed (**0**-None, **1**-Primary 2. Junior High School **3**-Secondary, **4**-Post-secondary, **5**-Post-graduate)	
3b. What is your occupation? Please specify: _____	
4. Do you earn any income? 1-Yes 2-No	
5. *If "Yes" please specify level of income per month* (**1**- <100GH¢, **2**-Between 100 – 250Gh¢, **3** – Between 251-500Gh¢, **4** – Above 500Gh¢)	
6. How do you normally pay your medical bills? (**1**-Self i.e. from personal income, **2** – Paid for or assisted by relatives, **3**-My present/former employer, **4** – National Health insurance, **5**-Other: please specify:_____)	
7. Do you currently smoke **1**- Yes **2**-No 7a. If you currently smoke, how many cigarettes do you smoke per day? 7b. If you do not currently smoke, did you smoke in the past? **1**- Yes **2**-No	
8. Do you use a vaginal herbal preparation? **1**-Yes **2**-No	
9. How many pregnancies have you had in the past? _____ 9a. How many of these pregnancies resulted in stillbirth? _____ 9b. How many of these pregnancies resulted in miscarriage/abortion? _____	
10. How many children do you have? _____	
11. What is your Relationship status? (**1**- Married, **2**- Living with Partner but not Married, **3** –Have a Steady Partner but not living together, **4** –Divorced, **5** –widow, **6**- Single,**7**- Other: please specify:.....)	__
12a Have you ever had sexual intercourse before? (**1**-Yes **2**-No) 12b. *If yes, Age at first sexual intercourse* _____	
If you ever had sexual intercourse before, please continue with the following questions, otherwise continue with part B.	
13. How many sexual partners have you had? _____	
14. Any sexual partner had more than one partner during lifetime **1**-Yes **2**-No **3**-Don't know	
15. What is your current contraceptive? **0**-None **1**-Pill **2**-Injectable **3**-Condom 4-Norplant/Jadelle **5**-IUCD **6**. Abstinence **7**-Other (Specify_____)	

B. Medical history

1. Have you ever been diagnosed with any of the following?	1-Yes	2-No	3-DK
i. Hypertension	_____	_____	_____
ii. Diabetes	_____	_____	_____
iii. Asthma	_____	_____	_____
iv. Sickle-cell	_____	_____	_____
v. Cancer	_____	_____	_____
vi. HIV/AIDS	_____	_____	_____
vii. Other 1 (please specify_____)	_____	_____	_____

If you have HIV/AIDS,
1a. How long has it been since diagnosis (in years and/or months)? _____/_____(yrs/mnths)
1b. Are you on antiretroviral medication?
***1**-Yes; for how long? _____/____(yrs/months) **2**-No **3**-Don't Know*
*1c. Are you symptomatic? **1**-Yes **2**-No **3**-Don't Know*
*1d. Have you been on any Antibiotics? **1**-Yes **2**-No **3**- Don't Know*
1e. if "Yes" name of Antibiotic? _____
1f. For how long have you been on Antibiotic? _____

2a. Last menstrual period [LMP] _____/___/__ ___ (*DD/MM/YY*)
2b. Interval since LMP
1-Less than 4 months **2**- 4 to 12 months **3**- More than 12 months **4**-Does not remember

3. Post-coital spotting or bleeding **1**-Yes **2**-No

4. Do you have or ever had [Select all that apply]
1-Pelvic pain with vaginal discharge **2**-Abnormal offensive vaginal discharge **3**-Vulvar ulcer/sore **4**-Vulvar Warts **5**-None of these

5. Current partner/spouse ever had [Select all that apply]
1- Pain on urination **2**-Abnormal offensive urethral discharge **3**-Penile ulcer/sore **4**-Penile warts **5**-None of these **6**- Don't Know

6. Former partner/spouse ever had [Select all that apply]
1- Pain on urination **2**-Abnormal offensive urethral discharge **3**-Penile ulcer/sore **4**-Penile warts **5**-None of these **6**-Don't Know **7-**Not applicable

7. Mother or Sister has or had cervical cancer **1**-Yes **2**-No **3**-Don't know

8. Have you ever been screened for cervical pre(cancer)? **1**- Yes **2**-No

If Yes;
8a. *How many times have you been screened in the past? _____ times*
8b. When were you last screened? ____/___/___(DD/MM/YY)
8c. If date is unknown, please indicate how long ago in years or months:____ years _____months
*8d. Have you ever had an abnormal pap smear? **1**- Yes; At the last screening? yes/no*
***2**-No*
***3**-Don't know*
*8d Have you ever had a biopsy? **1**- Yes; please briefly state the biopsy results_____*
***2**-No*
***3**-Don't know*

C. Presenting complaints (Briefly describe) [Applies only to patients screened in hospital]

Specific symptoms	Duration in months				Severity grade*			
	<1	**1-3**	**4-6**	**>6**	**1**	**2**	**3**	**4**
i. Bleeding Per Vaginam	___	___	___	___	_	_	_	_
ii. Painful intercourse	___	___	___	___	_	_	_	_
iii. Post-coital bleeding	___	___	___	___	_	_	_	_
iv. other:_____	___	___	___	___	_	_	_	_

*Severity grade: **1 - Mild** i.e. present with no effect on normal activities, **2- Moderate** i.e. minimal tolerable effect (symptomatic relief would suffice), **3- Severe** i.e. having significant effect on normal activity, **4-Serious** i.e. incapacitating, requiring hospitalization or potentially life-threatening.

D. Cervical cancer awareness

1. Have you heard about Cervical Cancer? *(**1**-Yes, **2**-No)*	__
1a. If Yes, how did you hear about **1** -Was just told about it, **2** -In this hospital **3** -Radio/TV **4** -Other (Please sp:_____)	
1b. If Yes, how long ago did you hear about it? ***1**- Less than 6 months **2** - Between 6 to 12 months **3**-More than 12 months*	
2. If there were facilities for screening for early detection, would you come regularly for check-up? ***1**-Yes, **2**-No*	
3. *If Yes, how much will you be willing to pay for such a service?* **1**- <10GH¢, **2**-Between 10 - <20Gh¢, **3** - Between 20 - <30Gh¢, **4** – at least 30Gh¢	___
4. Is your partner/husband aware of Cervical Cancer? **1**-Yes **2**-No **3**-Don't Know	__
5. Is your partner/husband aware that you are coming for screening for Cervical Cancer? **1**-Yes **2**-No	
6. Does your partner/husband approve of your undergoing this Cervical Cancer screening? **1**-Yes **2**-No **3**-Don't Know	

E. Acceptability of Evalyn® brush

1. Where sample was taken: 1-Clinic 2-CHPS Compound 3-Home
2. Who took the sample 1-Self-Unsupervised 2-Self-Supervised 3-Health worker
3. If you took the sample by yourself, please indicate how easy or difficult it was to use the Evalyn® brush for self-sampling? **1**-Very Easy **2**-Easy 3-Difficult **4-**Very difficult
4. If you took the sample by yourself, please indicate how comfortable you felt collecting your own sample with the Evalyn® brush ? **1**-Very Comfortable **2**-Somewhat comfortable **3-**Somewhat uncomfortable **4**-Very uncomfortable **5**-not applicable
5. If the sample was taken by a health worker, how comfortable was it? **1**-Very Comfortable **2**-Somewhat comfortable **3**-Somewhat uncomfortable **4-**Very uncomfortable **5**-Not applicable
6. Prior to this screening, had a health professional ever taken your sample during a pelvic examination? **1**-Yes **2**-No 6b. *If you answered "Yes",* how comfortable did you feel when the health professional collected your samples at your last pelvic exam? **1**-Very Comfortable **2**-Somewhat comfortable **3**-Somewhat uncomfortable **4-**Very uncomfortable **5**-Don't remember
7. If the Evalyn® brush works as well as going to the doctor, would you get checked more often, less often or about the same? 8-More often **2**-The same **3**-Less often.
9. If both sampling by speculum with brush and by sampling with Evalyn® brush can determine your risk of cervical cancer equally, which one would you prefer? **1-**Sampling with speculum and brush **2**-Sampling with the self sampler/ Evalyn® brush

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Signature Date