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A Study of Genital Human Papillomavirus (HPV) and Evaluation of HPV Testing for Cervical Cancer Screening in Women from the Eastern Cape Province, South Africa

By

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PREFACE AND DECLARATION

This thesis's experimental work was performed in the Institute of Infectious Disease and Molecular Medicine at the University of Cape Town, from October 2017 to November 2019, under the supervision of Anna-Lise Williamson, Zizipho Z.A Mbulawa and Tracy L. Meiring.

I Ongeziwe Taku, declare that the work described in this thesis is my original work, and has not been submitted in any form of diploma or degree at any university. Where use has been made of others' work, it has been acknowledged in the text and reference.

Student: Ongeziwe Taku (TKXONG001)

PLAGIARISM

"This thesis has been submitted to Turnitin software to check for similarity and originality. I confirm that my supervisor has seen my report and any concerns revealed with supervisor."

Declaration on the Inclusion of Publications in a PhD Thesis

I confirm that I have been granted permission by the University of Cape Town's Doctoral Degrees Board to include the following publication(s) in my PhD thesis, and where co-authorships are involved, my co-authors have agreed that I may include the publication(s). This study resulted into four publications. I was involved on the concept, design of the study, recruitment of participants, specimen collection, and specimen storage. I performed all the experiments for the study. The analysis and interpretation of data was done by Ongeziwe with the help of supervisors. The first drafts of the manuscripts was written by Ongeziwe. All authors were actively involved in the interpretation of the data, revision of the manuscript and approved the final version of the manuscript to be submitted.

1. **Ongeziwe Taku**, Charles B. Businge, Mana L. Mdaka, Keletso Phohlo, Wisdom Basera, Mirta Garcia-Jardon, Tracy L. Meiring, Ulf Gyllensten, Anna-Lise Williamson, Zizipho Z.A. Mbulawa (2020). Human papillomavirus prevalence and risk factors among HIV-negative and HIV-positive women residing in rural Eastern Cape, South Africa. *International Journal of infectious diseases*. https://doi.org/10.1016/j.ijid.2020.02.051. (published)

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4. **Ongeziwe Taku,** Adrian Brink, Tracy Meiring, Keletso Phohlo, Charles B. Businge, Zizipho Z.A. Mbulawa, Anna-Lise Williamson (2021)**.** Detection of sexually transmitted pathogens and co-infection with Human papillomavirus in women residing in rural Eastern Cape, South Africa. *PeerJ*. https://doi.org/10.7717/peerj.10793. (published)

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Signature: **Date:** 22/02/2021

DEDICATION

I dedicate this thesis to my mother (Nanziwe Taku), my late grandmother (Mahadi Florence Taku) and my family.

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I would like to express my thanks to my supervisors Prof Anna-Lise Williamson, Dr. Zizipho Z.A. Mbulawa and Dr. Tracy L. Meiring, for their assistance, guidance, encouragement, and constructive comments throughout my project.

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- **Swedish collaborators**

Prof Ulf Gyllensten: Principal Investigator in Sweden- Management and co-ordination of Project in Sweden.

Dr. Inger Gustavsson: Responsible for the validation and transfer of the hpVIR real-time test from Sweden to the University of Cape Town.

- **University of Cape Town collaborators**

Prof Lynette Denny: To ensure harmonization with other cervical cancer screening in the Eastern Cape Province.

A/Prof Jennifer Moodley: Responsible for questionnaire development and health systems aspects of the project.

- **Walter Sisulu University collaborators**

Dr. Charles B. Businge: Responsible for the application for local ethics approvals, participate in development of questionnaires, management of the study nurse, specimen collection, specimen storage, Pap smear and histology results.

Prof Mirta Garcia-Jardon: To ensure the Pap smear and histology is performed in all the clinical specimens and results made available to the project. Storage of specimens before shipping to University of Cape Town.

Keletso Phohlo: To ensure that Pap smear is done in all clinical specimens and the results are made available to the project.

I thank the staff of both study sites (Mbekweni Community Clinic and Nelson Mandela Academic Hospital Gynaecology Outpatient Clinic), the study nurse (Sr Virginia Maqoga), the research assistant (Luviwe Lutotswana) and women who kindly participated in the study.

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ABBREVIATIONS

AGC-NOS	Atypical glandular cells not otherwise specified
AIDS	Acquired immunodeficiency syndrome
ART	Antiretroviral therapy
ARV	Antiretroviral
ASC-H	Atypical squamous cells-cannot exclude HSIL
ASC-US	Atypical squamous of undetermined significance
ASIR	Age-standardised incidence rate
ASMR	Age-standardised mortality rate
ASR	Age-standardised incidence rate
BV	Bacterial vaginosis
CI	Confidence intervals
CIN	Cervical intraepithelial neoplasia
COX	Cyclooxygenase-prostaglandin
CT	*Chlamydia trachomatis*
DNA	Deoxyribonucleic acid
EC	Eastern Cape
EVA	Enhanced Visual Assessment
FDA	Food and Drug Administration
HC-2	Hybrid capture 2
HIV	Human immunodeficiency virus
HMBS	Hydroxymethylbilane synthase
HPV	Human papillomavirus
HREC	Human research ethics committees
HR-HPV	High-risk human papillomavirus
HSIL	High squamous intraepithelial lesions
HSV	Herpes simplex virus
CIN	Cervical intraepithelial neoplasia
IARC	International agency research council
IQR	Interquartile range
K	kappa value
LEEP	Loop electrosurgical excision
LLETZ	Large loop excision of the transformation zone
LR-HPV	Low-risk human papillomavirus
LSIL	Low squamous intraepithelial lesions
MG	*Mycoplasma genitalium*
MH	*Mycoplasma hominis*
NG	*Neisseria gonorrhoeae*
NILM	Negative intraepithelial lesions or malignancy
OR	Odds ratio
PCR	Polymerase chain reaction
ref	Reference
RLU	Relative light unit
SCC	Squamous cell carcinoma
STIs	Sexually transmitted infections
TP	*Treponema pallidum*
TV	*Trichomonas vaginalis*
UCT	University of Cape Town
UP	*Ureaplasma parvum*
UU	*Ureaplasma urealyticum*
VIA	Visual inspection acetic acid
WHO	World health organisation

ABSTRACT

Introduction: Human papillomavirus (HPV) infection is an important public health problem facing black African women. Persistent infection with high-risk (HR) HPV types is the key factor for the development of cervical cancer. Coinfection of HPV with other sexually transmitted pathogens contributes to the progression of cervical cancer. Preventative measures including screening for and treating pre-cancerous cervical lesions as well as HPV vaccination have been implemented in parts of South Africa. However, in the rural Eastern Cape Province there is limited information on the prevalence of HPV and the HPV types associated with cervical lesions. Two cohorts were chosen to study HPV in the Eastern Cape (South Africa), a community clinic, and a referral hospital for treatment of cervical lesions. This study aimed at determining the prevalence of HPV, risk factors of HPV, coinfection of HPV with sexually transmitted pathogens and evaluate the performance of a number of HPV tests for HPV detection and cervical cancer screening.

The objectives of the study were:

- To investigate the prevalence of HR-HPV and factors associated with HR-HPV infection among women from rural Eastern Cape, South Africa.
- To investigate the distribution of HPV genotypes among women with cervical intraepithelial lesions according to HIV status from Eastern Cape Province, South Africa.
- To investigate HR-HPV prevalence and compare agreement between clinician-collected and self-collected genital specimens as well as two different HPV tests on clinician-collected samples.
- To investigate the prevalence of sexually transmitted pathogens and co-infection of with HR-HPV infection among women from rural Eastern Cape Province, South Africa.

Methods: A total of 741 participants were recruited from the Mbekweni Community Clinic (N=417) and the Nelson Mandela Hospital Referral Clinic (N=324) located in the OR Tambo municipality of the Eastern Cape Province. Clinician-collected cervical scrapes from women attending the Community Clinic were screened for HR-HPV prevalence and HR-HPV viral load

using Hybrid Capture 2 (HC-2, Qiagen Inc., Gaithersburg, MD; USA); Cervical clinician-collected and vaginal self-collected specimens of women with or without abnormal cytology from both study cohorts were also screened for HR-HPV infection using *hpVIR* real-time PCR. HPV typing of clinician-collected cervical specimens from women with cervical intraepithelial neoplasia grades 2 and 3 (CIN 2 / 3) was done using Direct Flow Chip HPV kit (Master Diagnostica, Spain).

Cervical specimens from the Community Clinic (N=205) were also tested for sexually transmitted infections (STIs) namely *Chlamydia trachomatis*: *CT*, *Haemophilus ducreyi*, Herpes Simplex Virus type 2, *Neisseria gonorrhoeae*: *NG*, *Treponema pallidum*, and *Trichomonas vaginalis*: *TV*) and pathobionts (*Ureaplasma* spp: *(UP*), *Mycoplasma genitalium*: *MG*, and *Mycoplasma hominis*: *MH*) using the STD Direct Flow Chip kit (Master Diagnostica, Spain).

The univariate and multivariate analysis was used to determine the correlation between HPV infection and potential behavioural risk factors using STATA 14.2 (Stata Corp, College Station, Texas). A chi squared test was used determine the difference in estimated HR-HPV prevalence between self-collected and clinician-collected samples. STIs prevalence and association with behavioural risk factors were analysed using GraphPad Prism v6.01 (GraphPad Software, Inc., San Diego, CA).

Results: Of the 417 women from the community clinic, HR-HPV prevalence was significantly higher in HIV-positive women compared to HIV-negative women (40.6%, 63/155 vs 21.4%, 56/262, $p<0.0001$). Women who were HIV-positive ($p<0.0001$), having one sexual partner in the past last month ($p=0.01$), three life-time sexual partners ($p=0.02$), at least four times frequency of vaginal sexual intercourse in the past last month ($p=0.004$) and having vaginal discharge currently/in the previous week ($p=0.01$) were significantly associated with HR-HPV infection. HIV-positive women had a significantly high HPV viral load compared to HIV-negative women (RLU/CO: median 0.390 (IQR: 0.009-3245.33) compared to 0.180 (IQR:0.08-2884.2); $p< 0.0001$).

Among women referred to Nelson Mandela Hospital with cervical intraepithelial lesions, HPV prevalence was observed to be significantly higher in HIV-positive than HIV-positive women (98.0% vs 89.1%, *p*=0.012). Similarly, HIV-positive women (65.3%, 96/147) had higher multiple HPV infections than HIV-negative women (47.8%, 22/46; *p*=0.034). HPV35 (23.9%), HPV58 (23.9%), HPV45 (19.6%), and HPV16 (17.3%) were the most frequently detected HPV types in CIN2, while HPV35 (22.5%), HPV16 (21.8%), HPV33 (15.6%), HPV58 (14.3%) were commonly detected in women with CIN3 regardless of HIV status.

HR-HPV prevalence in clinician-collected samples was equivalent to self-collected samples from both study sites, the community clinic (26.4% vs 27.9%, *p*=0.601) and the referral clinic (83.6% vs 79.9%, *p*=0.222). HR-HPV positivity between self-collected and clinician-collected samples showed an agreement of 86.9% for community clinic (k=0.669) and 91.4% for referral clinic (k=0.711). The distribution of HR-HPV genotypes was similar between self-collected and clinician-collected samples from both study sites. The agreement of HR-HPV genotypes between self-collected and clinician ranged from moderate to almost perfect (0.571-0.888). A majority of women reported a high positive response of acceptance for self-collection (community-based clinic: 77.2% and referral clinic: 83.0%). HR-HPV detection agreement between *hpVIR* real-time PCR and HC-2 was almost perfect (87.7%, k=0.754).

The prevalence of the six traditional STIs (*CT*, *TV*, *NG*, HSV-2, *TP,* and *Haemophilus ducreyi*) was high (22.9%, 47/205). *TV* was the most frequently detected STI (15.6%, 32/205). *UP* (70.2%, 144/205) and *MH* (36.6%, 47/205) were the most frequently identified pathobionts. Multiple infections/coinfections with more than two STIs/pathobionts was found in 52.7% (108/205) of women with *UP*/*MH* (26.9%) and *UP*/HPV (21.3%) the frequently identified coinfections. HR-HPV infection was significantly associated with HIV infection (*p*=0.017) and HSV-2 (*p*=0.026).

Conclusion: This study shows that HIV infection and sexual behaviour increased the risk of HPV infection among women from the community clinic. HIV-positive women had significantly higher HPV viral load and multiple HPV type infections compared with HIV-

negative women with or without cervical lesions. Since HIV positive women are at higher risk of HPV infection they need to continue to be screened more regularly for cervical lesions and treated when appropriate. In addition, the high prevalence of HPV in the community of HIV negative women indicates that a robust cervical screening programme is needed to implement the cervical screening policy of South Africa. Thus, the women get the allocated three cervical smears in a life time. Distribution of HPV types was similar among women with CIN2 & 3 with HPV35 being the most frequently detected HPV type regardless of HIV status. This highlights the importance for the inclusion of HPV-35 in the next generation of HPV prophylactic vaccines. The findings of this study add to the limited information on genital HPV infection in women from this province. Moreover, our data now acts as a baseline/reference data for future investigations. The data will also contribute to discussions on HPV testing as the primary screening strategy for cervical cancer and HPV vaccination in South Africa.

The *hpVIR* real-time PCR test between self-collected and clinician-collected specimens showed comparable agreement for the detection of HPV infection. The type-specific concordance between self-collected and clinician-collected showed moderate to an excellent agreement, indicating that self-collection can be utilised as the alternative screening tool for cervical cancer. The participants showed a high positive response for the self-collection method, indicating that introducing this method can positively impact the cervical cancer screening program. However, *hpVIR* real-time PCR is an *in-house* test which is not practical to introduce on a large scale in South Africa. Therefore, future research should be done to determine what other HPV tests could be done on these types of specimens.

Presently, syndromic management is used to treat STI at clinics in South Africa. The high prevalence of sexually transmitted pathogens necessitates the need to enhance the current screening methods for these pathogens.

CHAPTER 1: Introduction and Literature Review

1.0 The burden of cervical cancer

Globally, cervical cancer is the third most common cancer in women and the leading cause of cancer mortality among women from low- and middle-income countries including those in sub-Saharan Africa (Bruni et al. 2019a). Sub-Saharan Africa (Eastern Africa, Southern Africa, middle Africa, Western Africa) has a high overall age-standardised incidence rate (ASIR) of 27.6 per 100,000 women-years compared with Latin America (Central America, Caribbean and South America; 11.2 per 100,000 women-years) and Asia (Southern-Eastern Asia, South Central Asia, Eastern Asia and Western Asia; 11.9 per 100,000 women-years) (Bruni et al. 2019a). Of the sub-Saharan African regions, the ASIR for cervical cancer ranges from 26.8-43.1 per 100,000 women-years while the age-standardised mortality rate (ASMR) ranges from 21.1-30.0 per 100,000 women-years (Figure 1.1) (Bray et al. 2018; Arbyn et al. 2020; Bruni et al. 2019a).

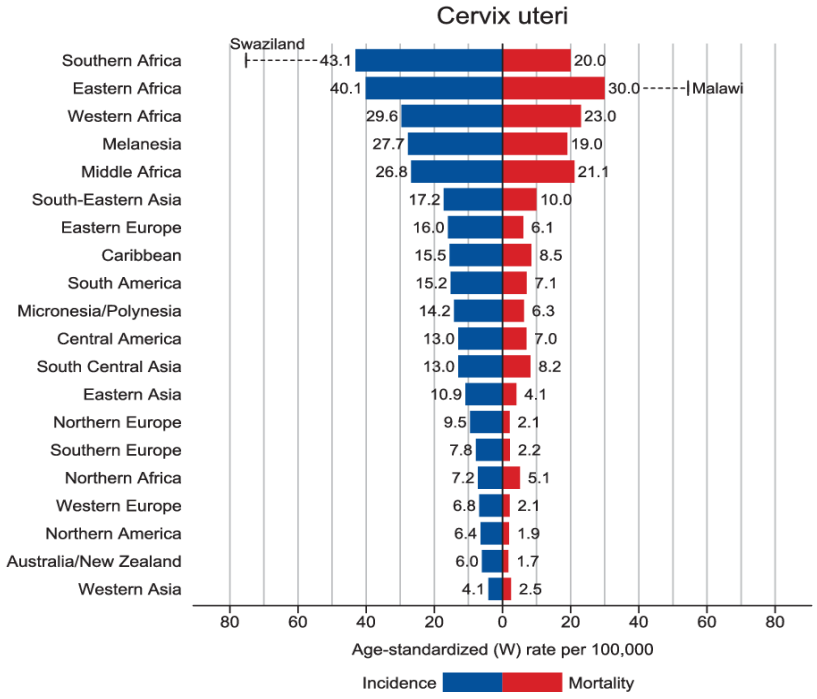

Figure 1.1: Age-standardised incidence and mortality rate of women with cervical cancer according to regions worldwide, taken from (Bray et al. 2018).

Southern Africa, Eastern Africa, and Western Africa are the top three high-risk regions, with eSwatini having the highest ASIR (75.3 per 100,000 women-years) and Malawi with the

highest ASMR (72.9 per 100,000 women-years) of cervical cancer compared to the rest of Africa (Figure 1.2) (Bray et al. 2018).

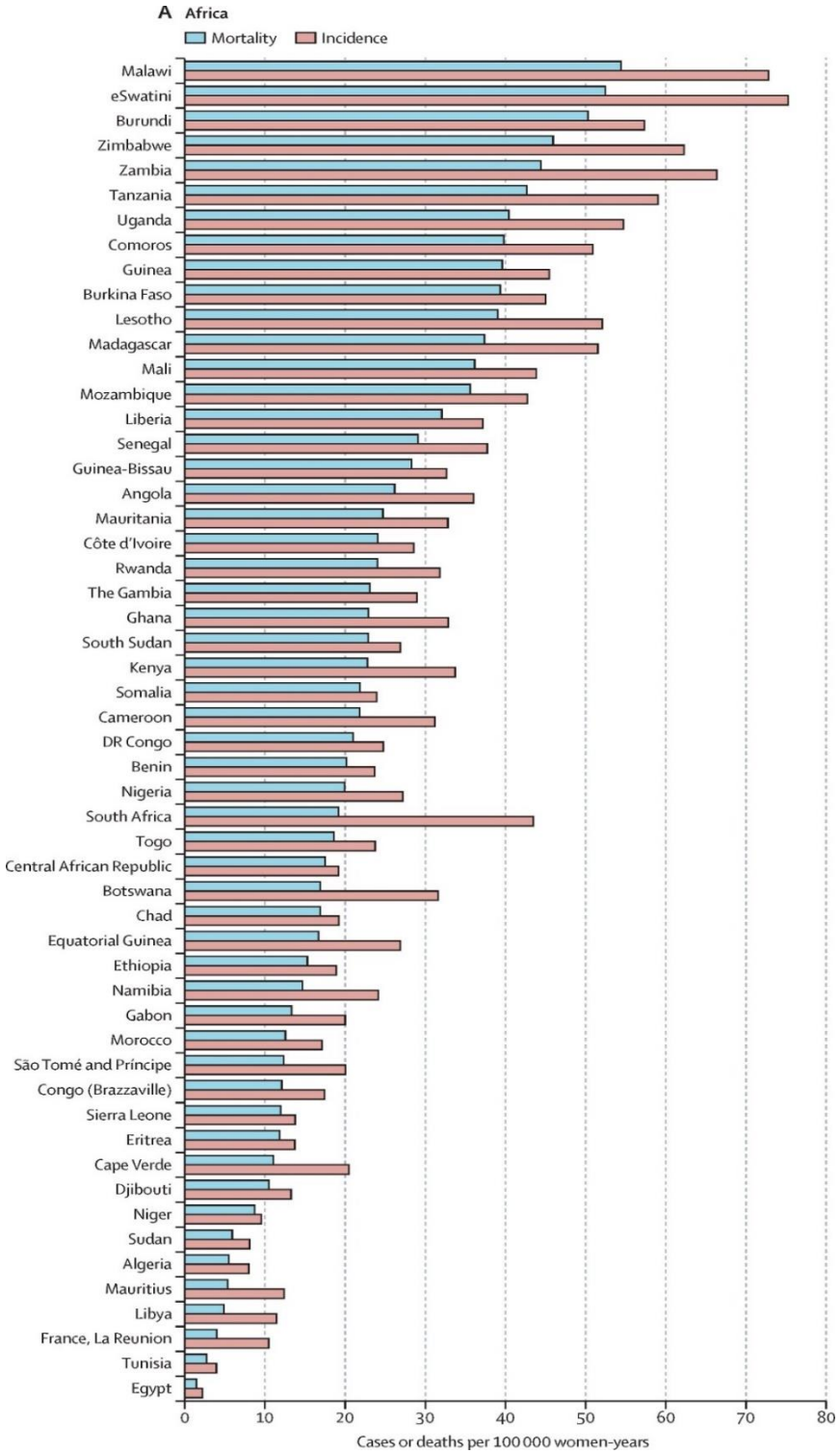

Figure 1.2: Age-standardised incidence and mortality rate of women with cervical cancer by African countries, taken from (Arbyn et al. 2020).

Among South African women, cervical cancer is the second most common cancer with 12, 983 new cases reported in 2018 and an ASIR of 44.4 per 100,000 women per year (Bruni et al. 2019b). However, in black women cervical cancer is the most common cancer (National Institute For Communicable Diseases Of South Africa, 2020). The ASIR of cervical cancer in the rural regions of the Eastern Cape region of South Africa ranged from 18.8 to 39 per 100,000 population during the period 1998 to 2012 (Figure 1.3) (Somdyala et al. 2020). Cervical cancer has become the most common malignancy in women in rural Eastern Cape, particularly in regions with limited resources (Somdyala et al. 2015a). Women in rural areas of the Eastern Cape are more prone to cervical cancer because screening is not fully implemented in rural areas and there high HIV infection. This is due to limited resources to implement screening programmes. Furthermore, it has been reported that factors including health inequity, unequal distribution of resources and low health-seeking behaviour have an impact on the increase of cervical cancer incidence in rural areas (Botha and Richter, 2015; Sibiya and Grainger, 2007; Ramathuba et al. 2016; Mosavel et al. 2009).

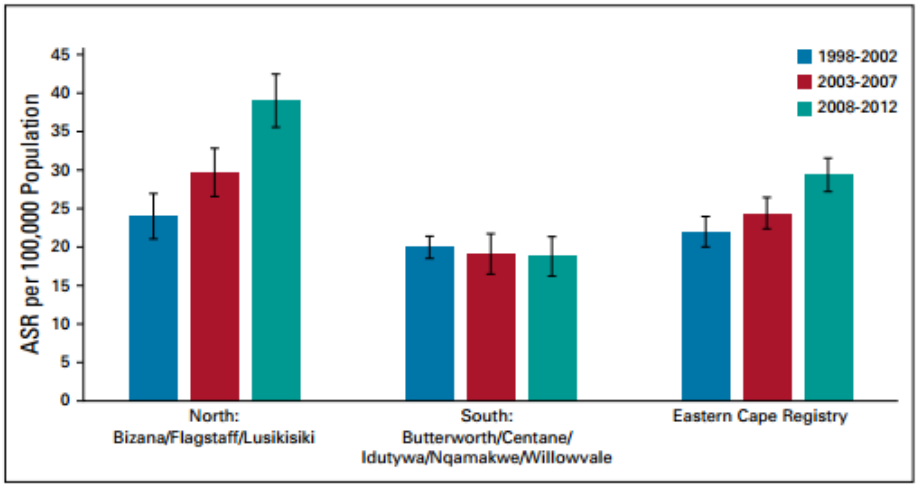

Figure 1.3: Age-standardized incidence rate (ASR) of cervical cancer between 1998-2012 in rural Eastern Cape regions, taken from (Somdyala et al. 2020).

The Eastern Cape Province cancer registry is a register of cancer patients living in rural areas and 99% of which are Black Africans (Somdyala et al. 2015b). Of the Eight municipalities present in Eastern Cape (Figure 1.4), the registry only covers two district municipalities (OR Tambo and Amathole District municipality) (Somdyala et al. 2015b) and eight magisterial

regions in (North region: Flagstaff, Bizana and Lusikisiki and the South region: Idutywa, Centane, Nqamakwe, Butterworth and Willowvale) (Somdyala et al. 2020; Somdyala et al. 2015a) (Figure 1.3). It collects and incorporates cancer data yearly from eight district hospitals, six referral hospitals and one pathology laboratory (National Health Laboratory services). Women are initially screened at their local hospitals/clinics and sent to the referral hospital for further evaluation if the cytology test is abnormal (Somdyala et al. 2020).

Figure 1.4: Eastern Cape map showing eight municipalities (https://municipalities.co.za/provinces/view/1/eastern-cape).

1.1 The burden of cervical intraepithelial lesions

Approximately 80% of cervical cancer cases are classified as squamous cell carcinomas. These cancers develop from the abnormal growth of the squamous epithelial cells in the transformation zone of the ectocervix known as cervical intraepithelial neoplasia (CIN) (Safaeian et al. 2007). CIN is regarded as a premalignant lesion and is classified into stages based on histology: CIN1 (mild dysplasia), CIN2 (moderate dysplasia), and CIN3 (severe dysplasia) (Figure 1.5) (Sellors and Sankaranarayanan, 2003; Bowden and Kyrgiou, 2020). CIN grades are used in the cervical screening programme to determine the risk of developing

cervical cancer (Waxman et al. 2012). CIN1 is considered a low-grade lesion, while CIN2 and3 are considered high-grade lesions (Waxman et al. 2012).

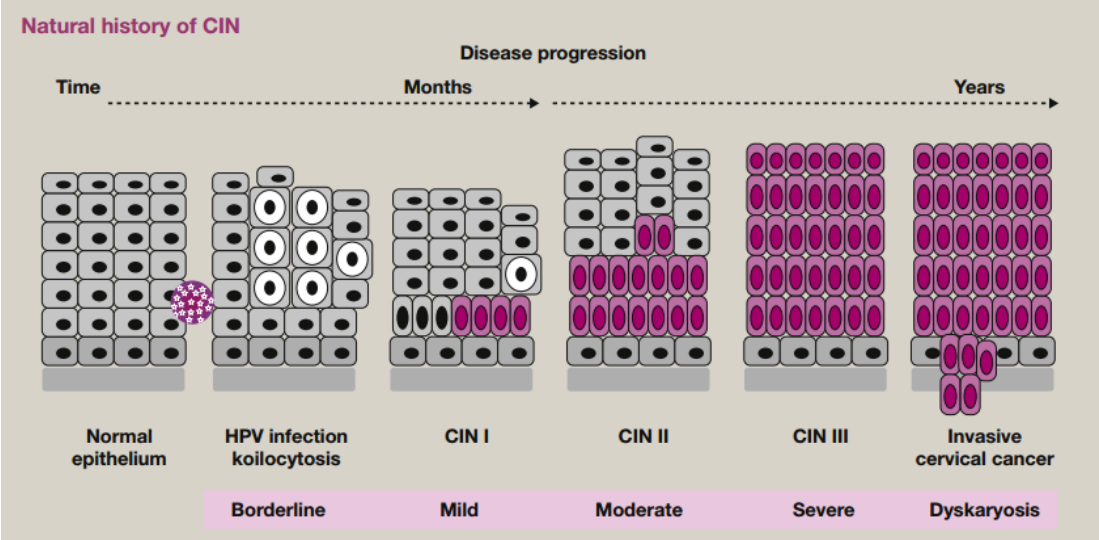

Figure 1.5: Natural history of cervical intraepithelial lesions showing the development of premalignant lesions stages, modified from (Bowden and Kyrgiou, 2020).

A worldwide systematic review reported an incidence rate of cervical intraepithelial lesions of <10% among women aged 30 years or more who had normal cytology at baseline and with follow-up for 5-127 months (Ting et al. 2015). According to MacDonald and colleagues (2014), the overall prevalence of cervical precancerous lesions was 5.0% for CIN1, 2.5% for CIN2, and 1.3% for CIN3 in both women aged 17-65 years recruited from a general population in Cape Town, South Africa. However, human immunodeficiency virus (HIV) positive women had a significantly higher prevalence of lesions than HIV-negative women e.g. 2% of HIV-positive women having CIN3 vs 1.1% in HIV negative women and 15.5% of HIV positive women having CIN2 vs 3.2% of HIV negative women (McDonald et al. 2014). It has been shown that the distribution of cervical intraepithelial lesion grades differs by age, with high regression rates and low progression rates observed among younger women compared to older women (Bekos et al. 2018; Chang et al. 2019; Munro et al. 2016). The peak of CIN1 is observed among women aged 20-30 years, while CIN2-3 is common in women aged >30 years (Vink et al. 2013; Ting et al. 2010). In younger women (<25 years), CIN1-2 has the likelihood to regress with a rate of 90% for CIN1 and almost 70% for CIN2 (Moscicki et al. 2010; Moscicki et al. 2004). A systematic review and meta-analysis showed that women age less than 30 years had a 60%

regression rate of untreated CIN2, and only 11% progressed to CIN3 within two years (Tainio et al. 2018). Among older women aged 35-65 years, the regression rate of CIN2 was 56%, and 16% progressed to CIN3 within six years (Wang et al. 2013). The higher-grade lesions (CIN2-3) are the most likely to progress to invasive cervical cancer. A clinical study from the Netherlands in women aged 30 years diagnosed with CIN2/3 or cervical cancer between 2000 and 2005 reported that 1.6% of CIN2-3 developed into cervical cancer within ten years (Vink et al. 2013). The study also estimated the median time to be 23.5 years for the development of CIN2-3 to cervical cancer (Vink et al. 2013). Human papillomavirus (HPV) infection is reported as a significant factor that plays a causal role in CIN's natural history.

1.2 Human Papillomavirus classification

HPVs belong to the *Papillomaviridae* family that includes 39 genera found in diverse species, including birds, mammals, and reptiles (snakes and turtles) (de Villiers et al. 2004; Bravo et al. 2010). Within the human papillomaviruses, 228 HPV genotypes have been identified belonging to five genera (alpha, beta, gamma, Nu, and Mu-papillomavirus) (Figure 1.6) (International human papillomavirus reference center 2020; Bernard et al. 2010; de Villiers 2013; Van Doorslaer et al. 2013). The genus alpha-papillomavirus contains the essential evolutionary branches of HPV types associated with severe human diseases (Plummer et al. 2016). There are 65 different alpha HPV genotypes identified (Mühr, Eklund, and Dillner et al. 2018). These HPV genotypes are broadly divided into mucosal types that infect the internal lining of organs such as anogenital, throat, or mouth, and the cutaneous types characterised by infection on the epidermis of the skin (feet and hands) (Burd 2003; Mistry et al. 2008).

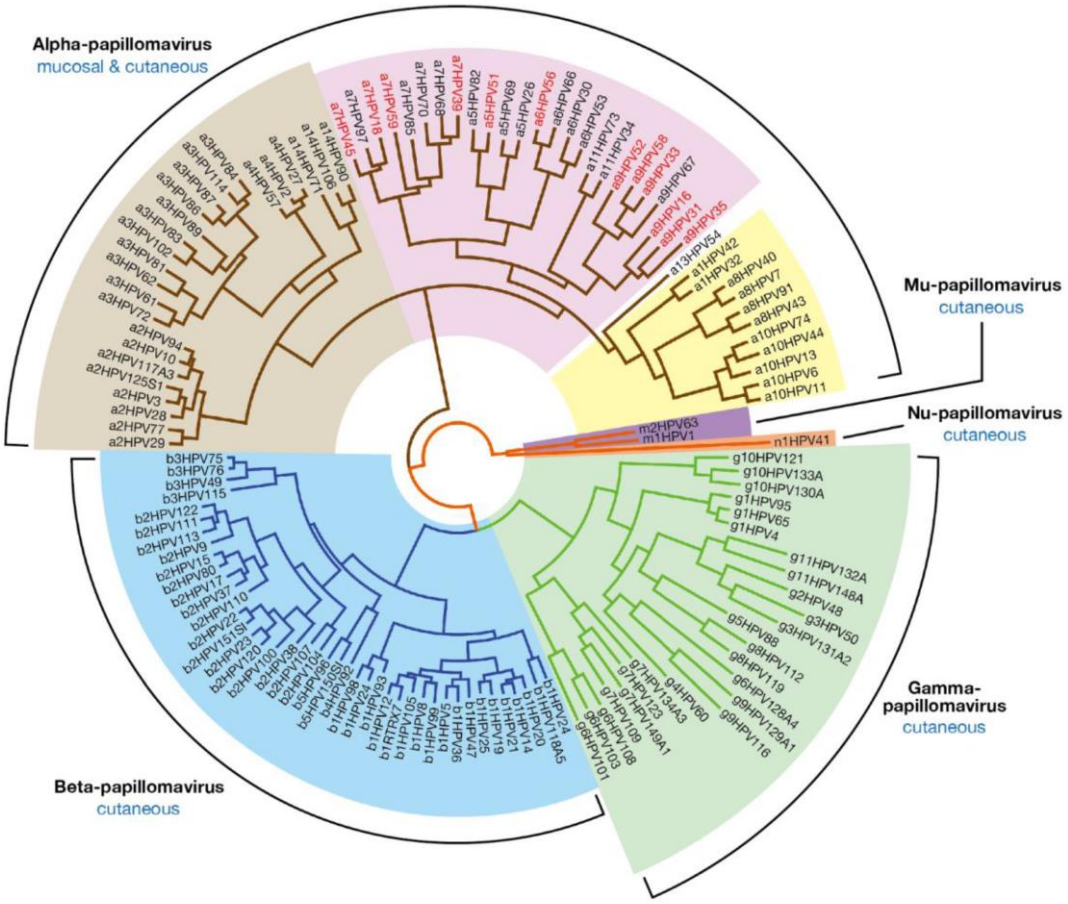

Figure 1.6: Evolutionary phylogenetic tree showing HPV types found from five genera, with alpha having the largest group of HPV types than other genera. The HPV types highlighted in red on alpha genera are known as human carcinogens, taken from (Egawa et al. 2015).

Mucosal HPV types are further classified into two groups: low-risk (LR), and high-risk (HR) HPV types. LR-HPV types are those types that are non-carcinogenic genotypes capable of causing genital warts. In contrast, HR-HPV types are associated with precancers or lesions and diverse types of cancer (cervical, vaginal vulvar, penile, anal, breast, oral, head and neck) (Bzhalava, Eklund, and Dillner 2015; Plummer et al. 2016; Michaud et al. 2014; Salman et al. 2017). Of the HR-HPV types, the International Agency for Research on Cancer evaluation (IARC) reported three high-risk groups (Group I, Group 2A, and Group 2B) that have carcinogenicity to humans (IARC 2012). Group I includes 12 high-risk types, for which there is strong evidence for a causal role in cervical cancer (IARC 2012). At the same time, Group 2A is classified as probably carcinogenic in humans with strong mechanistic evidence for a role in cervical

cancer. In contrast, Group 2B is classified as possible carcinogenic with limited evidence for causing cervical cancer (IARC 2012) (Table 1.1).

Table 1.1: HPV types classification into four groups according to the International Agency for Research on Cancer evaluation

HPV types classification	**HPV genotypes**
Low-risk HPV types	HPV6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, 83, 89
Group 1	HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59
Group 2A	HPV 68
Group 2B	HPV 26, 30, 34, 53, 66, 67, 69, 70, 73, 82, 85, and 97

Group 1: classified as carcinogenic high-risk HPV types; **Group 2A**: classified as probable carcinogenic; **Group 2B**: classified as possible high-risk HPV types, adapted from (IARC 2012).

1.3 The natural history of cervical HPV infection

1.3.1 The burden of HPV infection and HPV genotype distribution in Africa

Since HPV infection is the central aspect and the first step for developing cervical cancer diseases, it is crucial to determine the burden of HPV and HPV types present in the African population in order to know the HPV types involved in causing cervical cancer. Thus, this information helps to improve, implement suitable cervical cancer screening strategies, and observe the effect of HPV vaccines in the African population.

In women with normal cytology worldwide, the estimated prevalence of HPV is 12.0%, with the highest rates observed in African countries ranging between 12.8% to 57.3% (Ogembo et al. 2015; Bruni et al. 2010). The prevalence of HPV infection is related to age. The highest HPV prevalence is observed among 25-year-old women and it decreases as age increases. Cross-sectional studies done in South Africa, showed that the prevalence of HPV among adolescent and young women (16-24 years) ranges from 40% to 88.6 % (Adler et al. 2013; Ebrahim et al. 2016; Giuliano et al. 2015; Mbatha, Taylor, et al. 2017; Mbulawa et al. 2018; Menezes et al. 2018; Adler et al. 2014), while in older women it range from 16.0%-60.0% with or without HIV infection (Johnson et al. 2020; Mbulawa, Coetzee, and Williamson 2015; McDonald et al. 2014). The high prevalence in adolescent and younger women could be influenced by sexual behaviour, while in older women, it could also be the reactivation of a pre-existing or latent infection (Rositch et al. 2012; Winer et al. 2016).

A systematic review and meta-analysis performed among African women with abnormal cytology found that HPV prevalence was 74.2% in low-grade squamous lesions (LSIL), 84.8% in high-grade squamous lesions (HSIL) and 89.5% in invasive cervical cancer group (Ogembo et al. 2015). The predominant HR-HPV genotypes in South African women with normal cytology were reported to be HPV16 (11.0%), HPV51 (9.3%), HPV53 (9.1%), HPV58 (7.9%), HPV45 (7.5%), and HPV83 (2.6%) (Van Aardt et al. 2013; Allan et al. 2008). Among women with abnormal cytology, the most prevalent HR-HPV types were HPV52 (17.5%), HPV53, (15.8%), and HPV16 (12.3%) for LSIL and HPV16 (18.9%), HPV35 (18.9%) and HPV31 (11.3%) for HSIL (Allan et al. 2008). Figure 1.7 and 1.8 shows the HPV types in women with or with cervical cancer lesions from Africa and South Africa.

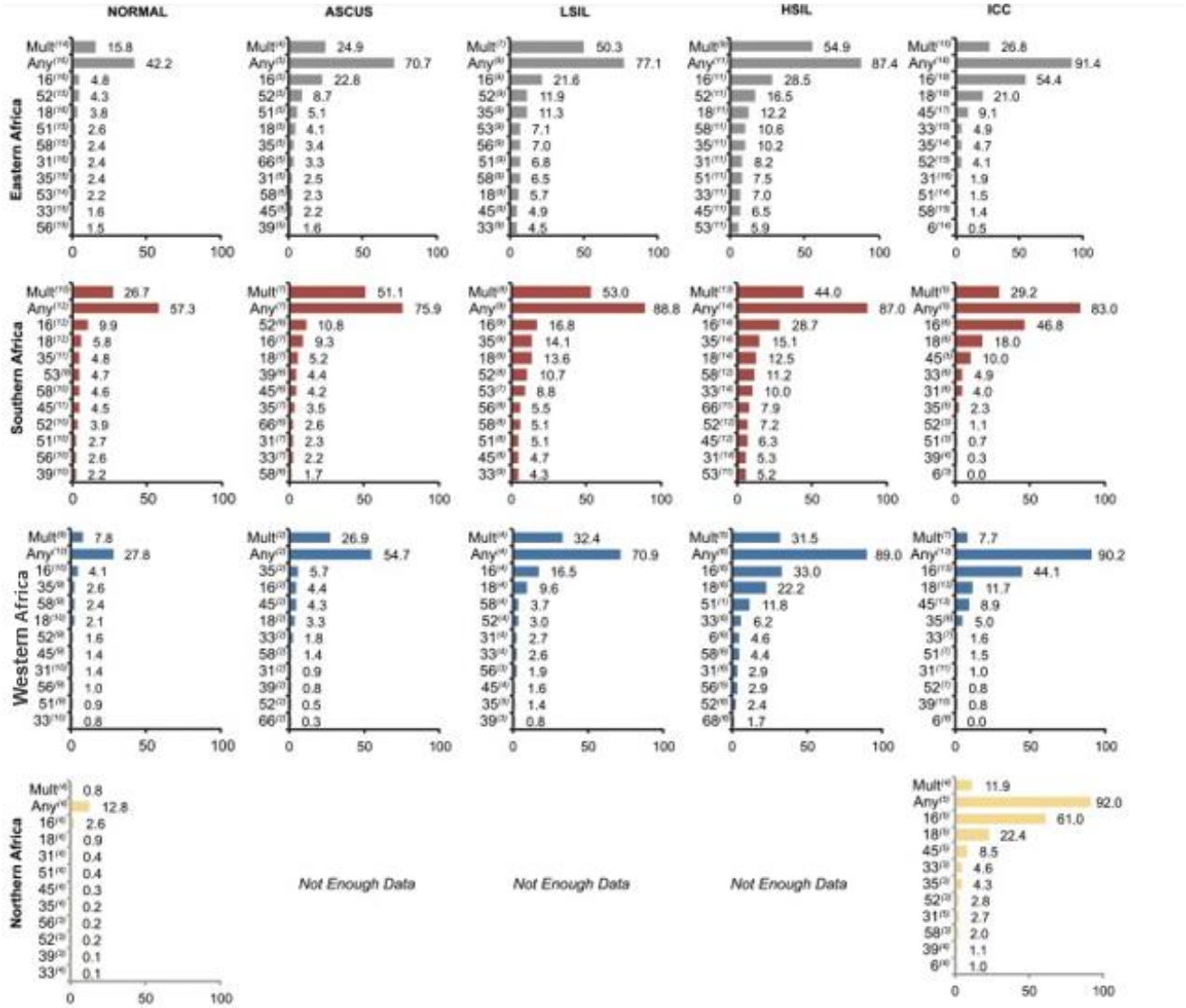

Figure 1.7: High-risk HPV types according to women with normal cytology, abnormal cytology, and invasive cervical cancer from Africa, modified from (Ogembo et al. 2015).

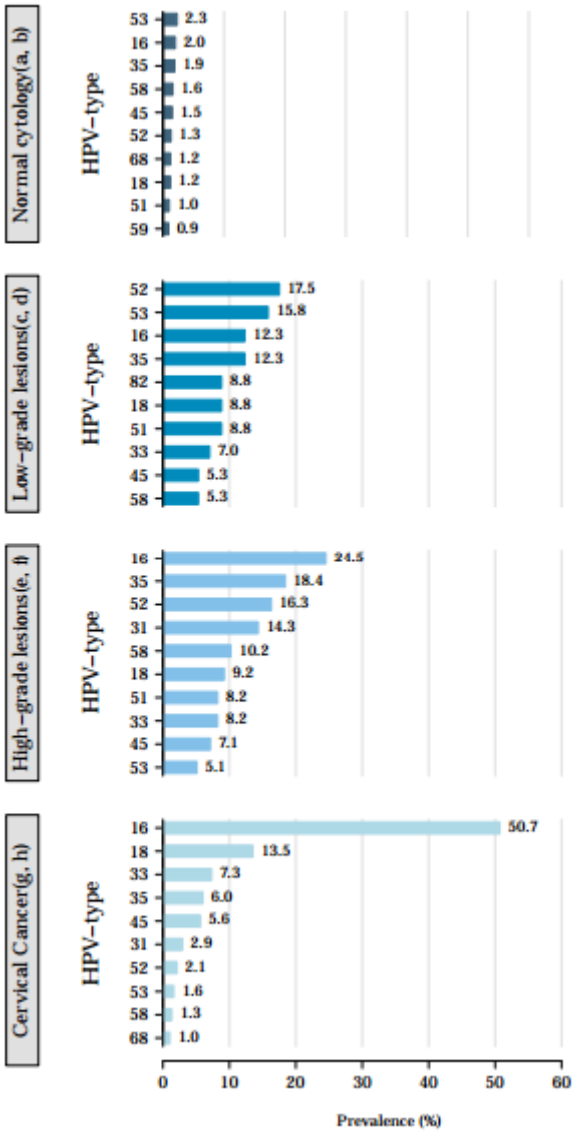

Figure 1.8: Distribution of high-risk HPV types in women with normal cytology, low-grade cervical lesions, high-grade cervical lesions, and cervical cancer from South Africa, modified from (Bruni et al. 2019a).

In cases of high-grade CIN2-3, the predominant HR-HPV types among HIV-negative women were found to be HPV16 (31.1%), HPV52 (22.2%), HPV31 (17.8%), HPV35 (17.8%) and HPV58 (17.8%) while HPV16 (32.9%), HPV58 (26.7%), HPV35 (25.3%), HPV51(23.1%) and HPV52 (21.3%) were common in HIV-positive women (Van Aardt et al. 2016). In the case of invasive cervical cancer, HPV 16 (50.7%) had the highest prevalence, followed by HPV18 (19.2%), HPV45 (10.1%), HPV35 (9.7%), HPV33 (5.0%) and HPV52 (4.5%) among women with invasive cancer from South Africa, Ghana and Nigeria (Denny et al. 2014). Figure 1.9 shows the global, cumulative proportion of cervical cancer cases attributable to HPV type (Arbyn et al. 2014).

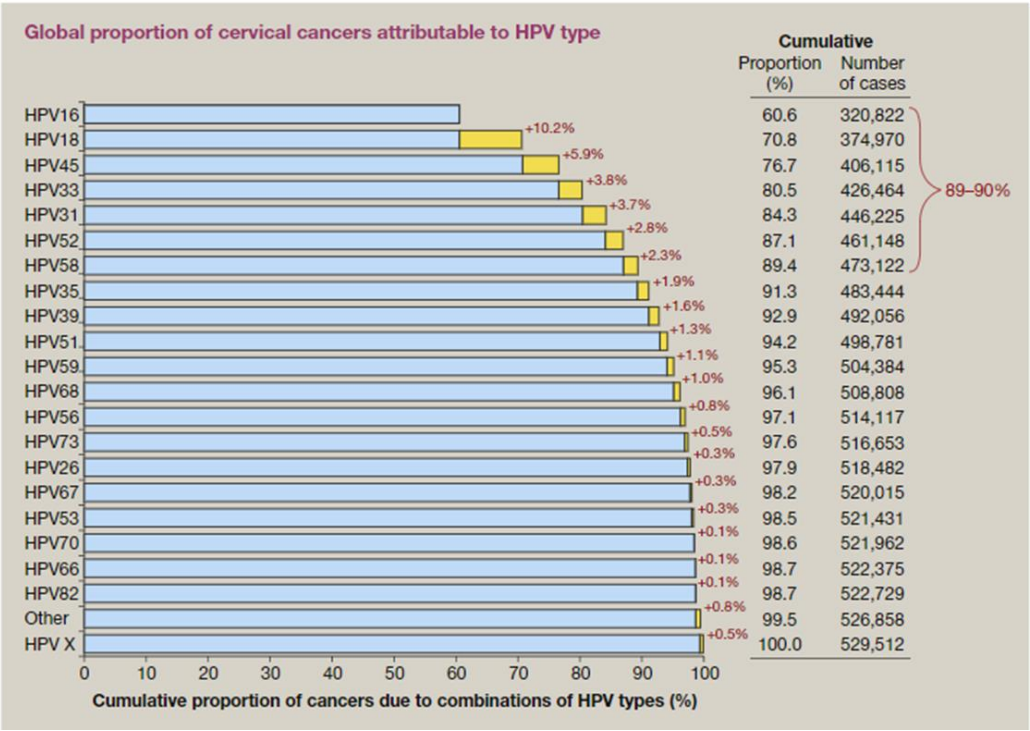

Figure 1.9: A distribution of high-risk HPV types associated with cervical cancer and cumulative proportion, taken from (Arbyn et al. 2014; Bowden and Kyrgiou 2020).

With vaccination being used as the primary strategy for preventing HPV infection and cervical cancer diseases. The HPV vaccines have been found to be harmless, well-tolerated, and effective in preventing cervical cancer diseases correlated with HPV types covered by the currently available vaccines (Lu et al. 2011). The HPV vaccination field is evolving with three prophylactic HPV vaccines developed and approved by United States Food and Drug Administration (FDA). These include bivalent HPV vaccine known as Cervarix (manufactured by GlaxoSmithKline) targeting HPV16 and HPV18; quadrivalent recognised as Gardasil (manufactured by Merck) targeting HPV types 16, 18, 11 and 6 (Harper and Vierthaler 2011; Markowitz et al. 2012). A 9-valent HPV vaccine (manufactured by Merck) comprises four same HPV types targeted by Gardasil and five additional HR-HPV types (33/31/45/52/58) (Joura et al. 2015). This vaccine targets 80%-90% of the HPV types associated with cervical cancer (Figure 1.8). Data from different countries have shown show that introduction of these HPV vaccines has effectively reduced the prevalence of HPV specific vaccine types, the incidence of genital warts, and high-grade cervical abnormalities (Kahn et al. 2012; Markowitz et al. 2013; Leval et al. 2012; Read et al. 2011; Crowe et al. 2014; Brotherton et al. 2011). These

vaccines are provided only younger women and boys who are not exposed to HPV infection and are available in many countries such as South Africa (Gardasil and Cervarix), Kenya (Gardasil and Cervarix) and Botswana (Gardasil) (Garland and Smith 2010).

1.3.2 New HPV infection

Genital HPV infection is most frequently reported viral infection occurring at higher rates compared to other HPV transmission routes and is associated with the development of genital diseases in different sites (Vogt et al. 2013; Insinga et al. 2008; Daling et al. 2002). There is a general agreement that genital HPV infection can occur through sexual contact and nonsexual routes such as skin to skin or environmental fomites (Liu et al. 2015; Sabeena et al. 2017). However, sexual contact is the predominant transmission route and is considered the primary pathway for spreading genital HPV infection (Kero and Rautava 2019). A South African study done among heterosexual couples who were followed-up for 6-24 months observed that female-to-male transmission rate of HPV infection (2.8 per 100 person-months) was 2.4-folds higher than male-to-female transmission (1.17 per 100 persons-months) (Mbulawa et al. 2013). However, the rate of new HPV infection was significantly higher in the penile samples (55.6 per 1000 person-months) compared to cervical samples within a follow-up of 24 months (33.8 per 1000 person-months) (Mbulawa et al. 2012). In a model-based study, the likelihood of transmission for the 14 HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68) was estimated to be 80% per any new sexual partner (Bogaards et al. 2010).

In the female genital tract HPV infects the cells around the normal cervix's transformation zone, which can result in abnormal cytology. Many HPV infections are short-term and asymptomatic, with most of them being eliminated by the host immune system (Jaisamrarn et al. 2013). It has been reported that new genital HPV infections can be detected within one year after the initial sexual interaction with an infected person (Moscicki, Ma, Jonte, et al. 2010). In a Tanzanian study, the acquisition of new HPV infections was 209 per 100 persons-years for any HPV infection and 63 per 100 persons-years for HR-HPV infection among adolescent girls who reported first sexual debut during the follow-up of eighteen months (Houlihan et al. 2016). Furthermore, another study on US women (18-24 years) reported an increase of HPV incidence from 28.5% within one year after sexual debut to almost 50%

during three-year follow-up (Winer et al. 2008). Although new HPV infections may be observed in all age groups, the acquisition of HPV is predominantly among adolescents and young women (Burger et al. 2017).

1.3.3 Persistent infection and clearance of HPV infection

After the HPV infection is established, it could be cleared or persist or become latent, and in rare cases progress to cancer (Figure 1.10). According to a study done by Winer and colleagues (2011) among young women (18-22 years), 90.6% of individuals infected with HPV cleared the infections within two years (Winer et al. 2011). However, other studies show that some HPV infections are more likely to be persistent (Thorsteinsson et al. 2019; Mollers et al. 2013). The duration of persistent HPV infection differs according to HPV type with an estimated median of 9.8 months for any HPV type and 8.4 months for LR-HPV types (Winer et al. 2011; Rositch et al. 2013). However, HR-HPV types are more likely to persist and have a longer duration of 1-2 years (Schiffman 2007; Winer et al. 2011). For example, follow-up studies have reported that HPV 16 was more likely to persist compared to other HR-HPV types within eighteen to twenty-four months (Mbulawa et al. 2012; Carcopino et al. 2011; Miranda et al. 2013). Host cell-mediated immunity is a factor that plays a significant role in the virus's clearance (Schiffman 2007).

HPV infection can enter the latent stage due to cellular immune response whereby the virus becomes undetectable, perhaps due to low viral load (Hammer et al. 2019; Maglennon, McIntosh, and Doorbar 2011) Immune compromised individuals such as HIV-positive women have a higher rate of latency and reactivation of latent HPV infections compared to HIV-negative women (Theiler et al. 2010). Older women are reported as more likely to be carriers of persistent HPV infection. A population-based cohort study showed that the risk of HR-HPV persistence was 2-fold higher among women aged 50-60 years compared to women aged 40-49 years and 3-fold higher than women aged 30-39 years (Mittal et al. 2017). According to Schlecht and colleagues (2003), women with persistent infection were likely to have LSIL (Schlecht et al. 2003). However, the majority of LSIL are more likely to regress, and less than 10% develop into HSIL (Ciavattini et al. 2019). Persistent or untreated HPV infection with co-factors such as sexually transmitted infections (STIs), parity, smoking and immunosuppression

increases the risk of progression of HSIL to invasive cervical cancer (Hoffman et al. 2016; Schiffman et al. 2007; Zhao et al. 2012).

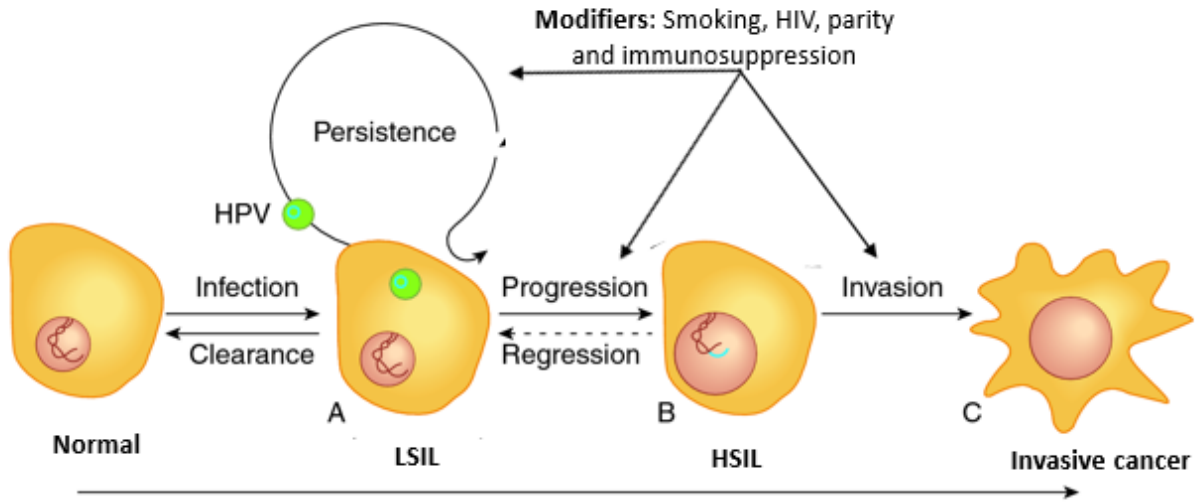

Figure 1.10: The natural history of human papillomavirus (HPV) infection and determinants involved in the progression of the HPV infection. Taken and modified from (Hoffman et al. 2016).

1.4 Cervical cancer screening

Cervical cancer can be prevented through cervical screening and treatment programmes (Koh et al. 2015). There are three significant possible approaches recommended in the screening guidelines for diagnosing cervical lesions and invasive cervical cancer, including screening with Papanicolaou smear test (Pap smear) (conventional cytology/liquid-based cytology), visual inspection with acetic acid (VIA) or HPV testing, colposcopy, and histology diagnosis (WHO 2013).

1.4.1 Cervical cytology and liquid-based cytology

The Pap smear test is a screening tool utilised to evaluate cervical cells to determine any abnormal changes by examining the cells using microscopy. These cytological abnormalities are classified according to the Bethesda system: atypical glandular cells not otherwise specified (AGC-NOS), atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells-cannot exclude HSIL (ASC-H), LSIL and HSIL (Solomon et al. 2002; Nayar and Wilbur 2015). Over the past 50 years, conventional cytology has been utilised as the standard

non-molecular screening method for cervical cancer in the screening programmes (Mitteldorf 2016; Saslow et al. 2012). The conventional cytology technique requires obtaining the cervical cells using a spatula and smear onto the slide (Gupta et al. 2019). Several studies reported that the conventional cytology test has significantly contributed to the reduction of the incidence and mortality rate of cervical cancer in Australia, England (Simonella and Canfell 2013), and the United States (Campbell et al. 2012; Tota et al. 2017). However, conventional cytology tests have a low sensitivity (23-55%) with high specificity (84-100%) to detect high-grade precancerous lesions (Khodakarami et al. 2011; Vahedpoor et al. 2019; Mayrand et al. 2007). The estimate of false-negative rates for cytology screening ranges between 13.8%-50% (Mayrand et al. 2007; Koonmee et al. 2017).

On the other hand, liquid-based cytology method is based on obtaining cervical cells using a cytology brush and transferring them into ThinPrep or SurePath solution (Gupta et al. 2019; Rozemeijer et al. 2017). Compared to conventional cytology, liquid-based cytology has several advantages. It is cost-effective, more accurate, and can be used for testing for HPV infection or staining for biomarkers (Gupta et al. 2019; Armstrong and Guest 2020; Del Pino et al. 2015). Moreover, liquid-based cytology samples contain more cervical cells due to the use of a cytobrush sampling device and have low rates of unsatisfactory specimens compared to conventional cytology (Liptak and Barnetson 2012; Ito et al. 2020). Developed countries such as Denmark, the United Kingdom, and the Netherlands have replaced conventional cytology with liquid-based cytology (Rask et al. 2014; Public Health England 2009). A population-based study done in the Netherlands reported a low incidence rate of invasive cancer with SurePath (44.6 per 100, 000 person-years) compared to conventional cytology (58.5 per 100,000) after the normal cytology test (Rozemeijer et al. 2017). Furthermore, a study done in Brazil among referral women reported significantly higher sensitivity with liquid-based cytology (1.43% for ThinPrep and 0.91% for SurePath) compared to conventional cytology (0.71%) for the identification of high-grade lesions (de Oliveira et al. 2020). However, other studies show similar performance between liquid-based cytology and conventional cytology with an adequate rate of 88.7% and 86.6%, respectively (Dhananjaya and Kumari 2017).

In South Africa, the general population's screening guidelines in the public sector recommend women of aged ≥30 years to have three Pap smears in their lifetime, at ten-year intervals. However, among HIV-positive women, cervical cancer screening begins at the age <30 years or at HIV diagnosis, and the policy recommends 3-year intervals if the cytology result is negative or annual screening if the cytology result is positive (National Department of Health 2017). In public sector the Pap smear test is free while women going to private sector they are required to pay.

1.4.2 Visual Inspection with Acetic Acid/Lugol's iodine

VIA is a simple, low cost and suitable method for cervical cancer screening (Sauvaget et al. 2011). VIA involves applying a solution containing 3-5% acetic acid to the cervix to identify precancerous lesions on the cervix and is viewed with the naked eye (Mabeya et al. 2012; Khan et al. 2015). VIA is easy to access and reduces multiple visits by combining screening with treatment on a single visit (Denny, Quinn, and Sankaranarayanan 2006). However, it has been shown to have low accuracy in other studies (Bhattacharyya, Nath, and Deka 2015; Sinha, Srivastava, and Srivastava 2018). In low-income countries such as Ghana, VIA has been included in the national screening guideline and is recommended for women 25-45 years of age (Ministry of Health 2017). A meta-analysis of studies done in sub-Saharan Africa showed that VIA's sensitivity and specificity differed by country, whereby the highest sensitivity for CIN2+ was observed in Tanzania (94.4%) while the lowest in Mali (65.0%) (Fokom-Domgue et al. 2015). However, the specificity was low in Zimbabwe (65.1%) and highest in Tanzania (98.2%) (Fokom-Domgue et al. 2015). VIA is reported to have a higher sensitivity (77.0%) than conventional cytology (59.0%) but lower sensitivity than liquid-based cytology (88.0%), while the specificity was observed to be similar (87.0% for VIA, 94.0% for conventional cytology, and 88.0% for liquid-based cytology) (Chen et al. 2012). VIA is negatively associated with adequacy among older women (Cremer et al. 2011).

1.4.3 HPV DNA testing

Since HR-HPV persistence is necessary for cervical cancer development, HPV DNA testing has become an alternative test for cervical screening. HPV testing depends on molecular methods

designed to detect nucleic acids of specific HPV genotypes (Poljak et al. 2012). Several HPV DNA tests have been used to test for HPV infection in the screening programmes. These include some of the commercial HPV DNA tests approved by the FDA for HPV testing, i.e., Hybrid Capture-2 (HC-2) (Qiagen, USA), Care HPV Test (Roche, USA), Cervista HPV Test (Hologic, WI), Cobas 4800 HPV Test (Roche, USA), Aptima HPV assay (Gen probe) and BD Onclarity (Becton Dickinson diagnostics, Sparks, USA) (Salazar et al. 2019; Poljak et al. 2012). Several developed countries have transitioned from cytology-based screening to HPV testing as the primary method for cervical cancer screening (Rebolj et al. 2019; Polman, Snijders, et al. 2019; Hortlund et al. 2018). There are currently different strategies implemented for HPV testing, including HPV DNA testing as the primary screening test, as a triage test to identify women who should be referred for further investigation or return to the standard screening modality, and co-testing (HPV and cytology at the same visit). Furthermore, the implementation status of these cervical screening strategies differs by country. Some of these are discussed in the sections below.

1.5 HPV DNA testing strategies

1.5.1 HPV DNA test as the primary screening/alone

HPV testing as the primary screening method has been implemented in developed countries (US and Australia) among women aged 30-65 years (von Karsa et al. 2015; Huh et al. 2015; Australian Government Department of Health 2017). Studies have shown that HPV tests alone are more effective and sensitive but less specific than Pap smear in identifying high-grade lesions (Sangrajrang et al. 2017; Tshomo et al. 2017; Agorastos et al. 2015). The cumulative incidence rate of CIN3 using HPV testing alone was significantly lower for negative HPV results (2.3 per 1,000) compared to 5.5 per 1,000 of a normal cytology test within four years (Ogilvie et al. 2018). A European trial of four studies showed that invasive cancer's cumulative incidence ranged from 3.3-18.6 per 100,000 population in HPV-negative women (Ronco et al. 2014). In contrast, for the cytology-negative test, it ranged between 7.9-27.0 within five years (Ronco et al. 2014).

The sensitivity of HPV testing using the FDA approved tests ranges from 82%-100%, with specificity between 61%-92.4% to detect CIN3 or higher (Rebolj et al. 2015; Phillips et al. 2015;

Ejegod et al. 2016; Cuschieri et al. 2015). The HPV DNA test protects cervical cancer cases by 60-70% compared to cytology testing (Ronco et al. 2014). HPV testing alone has a high negative predictive value, allowing cervical cancer screening intervals to increase to five years for a negative HPV test (Arbyn et al. 2012). However, compared to cytology screening, primary screening of HPV testing increases the number of women referred to colposcopy (Arbyn et al. 2012; Bains et al. 2019). Therefore, a single HPV test would not be useful for younger women as they are more likely to have a high regression rate of HPV infection (Bains et al. 2019).

In HIV-positive South African women, the sensitivity of HPV testing alone was observed to be significantly higher compared to cytology testing (83.1% vs 59.3%) but with lower specificity (66.4% vs 91.6%) (Kremer et al. 2019). A single HPV testing with the currently approved FDA tests is expensive, and for low-income countries, these costs would be out of reach. The estimated cost of HPV testing in South Africa for initial screening ranges from $36.50 to $64.83 USD (Lince-Deroche et al. 2015; Dreyer, Maske, and Stander 2019). Therefore, an inexpensive HPV test such as the careHPV test has been designed, and the cost per test is estimated to be ~$5 USD (Camel and Maza 2020).

1.5.2 Triage testing

The triage test is characterised as the strategy in which HPV testing is utilised to stratify women who primarily had low-grade cervical cytology abnormalities by identification of HR-HPV types (HPV16/18) (Kelly et al. 2011; Public Health Agency 2013; Wentzensen et al. 2016). Approximately 10-20% of women are referred for colposcopy/biopsy and treatment (Public Health Agency 2013). The triage screening approach eliminates HPV-negative women who are at low risk of precancerous lesions or cancer and identifies HPV-positive women with persistent infection at high-risk of cervical cancer lesions and who require to be referred to the colposcopy clinic for treatment (Wentzensen et al. 2016; Cox et al. 2013). This HPV strategy test decreases the time for women to return to routine recall and the multiple repeat of Pap smear test (Kelly et al. 2011). Also, the triage approach is useful for tests that lack specificity and leads to early identification of precancerous lesions and reduces overtreatment as low-income countries have with limited resources (Wentzensen et al. 2016; Cox et al. 2013). There are various triage strategies for HPV-positive women, such as p16/Ki-

67 dual stain cytology, HPV16/18, repeat cytology test, viral load, and viral methylation testing (Luttmer et al. 2016). Dual stain cytology is the identification of coexpression of p16, a tumor suppressor protein upregulated by HPV oncogene activity, and the cell proliferation marker Ki-67. Of these methods, only two triage test methods have been approved by FDA [HPV16/18 and cytology (threshold of ASC-US)] with screening intervals of three years (Gage et al. 2014; Saslow et al. 2012; Moyer 2012; Zaritsky 2019), while other methods are being evaluated (Luttmer et al. 2016; Wentzensen et al. 2016). These two triage tests have been approved in developing countries namely in USA, Australia Sweden, Norway and Netherlands (Huh et al. 2015; Australian Government Department of Health 2018; Saslow et al. 2012; Cuschieri et al. 2018)

In previous studies, using a single cytology test to triage women positive for HR-HPV infection revealed a sensitivity ranging from 52%-75.4% with specificity between 78%-85.6% among CIN3 or higher (Rijkaart et al. 2012; Dijkstra et al. 2014; Castle et al. 2011). A trial study done in Canada comparing the strategies of triage approach for women positive on HPV alone showed that HPV genotyping (HPV16/18^{+} or 16/18^{-}) with reflex cytology (Reflex cytology is the detection of HPV in a liquid-based Pap smear sample) yielded a high sensitivity (82.5%), moderate specificity (64.3%), and the highest rate of referrals (42.3%) (Isidean et al. 2017). However, the approved cytology triage with a threshold of ASCUS showed a low sensitivity (47.8%), high specificity (84.5%), and a low number of referrals (19.5%) (Isidean et al. 2017). A population-based study done among Mexican women showed that the triage approach of HPV16/18 with reflex cytology had a higher sensitivity (86.6%) to identify CIN2 or higher than liquid-based cytology (42.9%) but low specificity (34.0% vs 74.0% respectively) (Torres-Ibarra et al. 2019). The referral rate was 2-fold higher for HPV16/18 combined with reflex cytology (29%) than liquid-based cytology (12%) (Torres-Ibarra et al. 2019). An emerging triage strategy is a dual-stain cytology with HPV testing observed to decrease cervical cancer incidence by 36% and mortality by 40% (Tjalma 2017; Tjalma, Kim, and Vandeweyer 2017). The dual stain cytology negative test has been found to have a low risk rate for CIN2 or more compared to the negative cytology test within five years of follow-up (8.5% vs 12.3%, respectively) (Clarke et al. 2019). Furthermore, it has been observed that dual stain cytology has a low rate of referral women to colposcopy (Clarke et al. 2019). Therefore, dual stain

cytology is an attractive biomarker and safe for extension of cervical cancer screening intervals.

1.5.3 Co-testing

Co-testing incorporates both the HPV DNA test and cytology test to detect high-grade intraepithelial lesions at the same visit (Cuzick et al. 2015; Felix et al. 2016). The co-testing approach is reported as the safest screening method, has the probable of improving clinical outcomes and low risk of cervical cancer lesions for women who test negative on both screening tests (Ibáñez et al. 2020; Schiffman et al. 2018; Felix et al. 2016). However, co-testing has been found to be costly (Schiffman et al. 2018). Co-testing with a validated HPV DNA test was approved in 2003 by the FDA and included in the routine screening for women aged between 30-65 years in developed countries like the USA (Saslow et al. 2012; Katki et al. 2011; Gage et al. 2014; Wright and Schiffman 2003). Since the approval of co-testing, this approach's uptake has increased 9-times from 8.9% to 78.4% between 2006-2013 in developing countries (Silver et al. 2018). A retrospective study showed that the cumulative risk of developing high-grade lesions differs, with women who are HPV-positive/cytology-positive having the highest risk of 19.2%, followed by HPV-negative/cytology-positive (7.9%) and HPV-positive/cytology-negative (3.1%) (Ge et al. 2019). Women who tested negative on both screening tests are required to repeat screening after five years (Castle 2015). However, those positive on the HPV test but negative on cervical cytology are requested to repeat the co-testing after one year (Saraiya et al. 2014; Castle 2015). The advantage of co-testing is that it uses the combination of a highly sensitive test (HPV testing) and a highly specific test (cytology screening) to identify women with cervical lesions (Tota et al. 2014; Tota et al. 2010).

A study conducted in Spain among older women (40 years or more) found that the CIN2/3 incidence rate was 0.4% within five years and 1.3% within nine years for women both negative for cytology and HPV testing at baseline (Ibáñez et al. 2020). Therefore, the authors suggest that the low incidence rate shows that the extension of cervical screening intervals is safe for women with negative co-testing results at baseline (Ibáñez et al. 2020). In women with CIN2, the co-testing sensitivity of detecting HPV infection was 72.5%, with a 96.5% specificity, a

positive predictive value of 97.8%, and a negative predictive value of 62.5% (Choi et al. 2016). For CIN3, co-testing shows low sensitivity of 60% in women with CIN3 with a high specificity of 96.5%, a positive predictive value of 97.8%, and negative predictive value of 63.6% (Choi et al. 2016). Compared to HPV testing alone, co-testing had higher sensitivity (98.8% vs 94.0%) in detecting CIN3 or higher (Blatt et al. 2015). Furthermore, the incidence rate of cervical cancer among women with negative results of co-testing was observed to be lower (3.2 per 100,000) compared to HPV-negative result alone (3.8 per 10 000) and cytology testing (7.5 per 100,000) after five years intervals (Katki et al. 2011). However other studies show different findings. For example, a study done by Liang and authors (2020) showed that the co-testing and HPV alone had similar sensitivity, with co-testing having lower specificity than HPV alone, resulting in high number of false positive tests and referrals to colposcopy. According to the authors, this indicate that co-testing is less beneficial and that HPV test as a primary screening offers a balance of benefits and harms compared to co-testing (Liang et al. 2020).

1.6 Strategies for managing HPV-positive women

1.6.1 Colposcopy

In South Africa, women with ASC-H and HSIL on cytology testing are usually referred to the colposcopy clinic. The ongoing referrals and treatment for cervical cancer is free in the public sector. Colposcopy is a standard procedure used to view the cervical transformation zone, identify the cervical cancer lesions, and evaluate them for histology diagnosis through biopsy sampling (Jeronimo and Schiffman 2006; Wentzensen et al. 2015). A biopsy allows one to determine the diagnosis of precancerous lesions that will require treatment to prevent further progression (Wentzensen et al. 2015). Colposcopy has been revealed to have low sensitivity and interobserver or intraobserver agreement to detect high-grade cervical cancer lesions (Massad et al. 2009; Ferris and Litaker 2005). It has been previously reported that it is challenging to implement conventional colposcopy in low-income countries (Xue, Ng, and Qiao 2020). Therefore, digital colposcopes have been designed and are more sensitive, accurate, inexpensive, and easy to use in villages (Newman et al. 2019; Basu et al. 2016). The Enhanced Visual Assessment (EVA) system is one of the emerging portable digital colposcopy devices with smartphone features such as a customised camera and enhanced smartphone device light for visualization (Peterson et al. 2016). The EVA system has software that

monitors and evaluates the cervix area (Peterson et al. 2016). EVA's advantages include low cost, a better-magnified image of the cervix area, high-quality digital images, and images that can be stored and sent electronically to investigate further cases that are challenging to evaluate (Peterson et al. 2016).

Women identified with high-grade cervical lesions on histology diagnosis receive treatment. Various treatments are used, such as loop electrosurgical excision (LEEP), cold knife colonisation, large loop excision of the transformation zone (LLETZ), cryotherapy, and thermocoagulation (Massad et al. 2013; WHO 2014a; Chamot et al. 2010). Cryotherapy and LEEP are the most frequently used treatments for precancerous lesions in developing countries (Chamot et al. 2010). WHO recommended that cryotherapy be used in the general population and high-risk population to treat high-grade lesions (WHO 2014a). A systematic review and meta-analysis reported that LEEP has a significantly lower risk of persistence within six months and recurrence within one year of follow-up biopsy than cryotherapy (D'Alessandro et al. 2018). A randomised study done among HIV positive South African women reported that both cryotherapy and LEEP effectively reduced CIN2+ by more than 70% within one year. Furthermore, LEEP's efficacy was observed to be 81.5%, while that of cryotherapy ranged between 72.8% among women with precancerous lesions (Smith et al. 2017).

1.6.2 Screen-and-treat

A screen-and-treat approach allows women to be screened and receive treatment (for women positive for HR-HPV types) in a single visit. The screen-and-treat strategy eliminates the delays that require further evaluation before treatment, such as colposcopy and cervical biopsy tissue for women who test positive for HR-HPV infection (Kuhn et al. 2010; Denny et al. 2005). Low-income countries have adopted the screen-and-treat strategy and is reported to reduce CIN1-2 by 88% and CIN3 by 70% (Luciani et al. 2008). In a South African study, screen-and-treat using the HPV test was evaluated as an effective approach for managing HPV-positive women and reduced high-grade lesions by 2-fold compared to VIA (Denny et al. 2005). Moreover, the screen and treat approach resulted in a significantly lower rate of CIN2+ using the HPV test (77%) and VIA (37%) compared to women who received delayed evaluation

within six months (Denny et al. 2005). The advantage of the screen-and-treat approach is that it is cost-effective and reduces the number of appointments and losses to follow-up (Goldie et al. 2005; Waxman 2016). However, this approach is reported to be more prone to overtreatment (Goldie et al. 2005; Waxman 2016).

The screen-and-treat approach uses cryotherapy or thermocoagulation to treat high-grade lesion or HR-HPV-positive women with genotype 16/18. Thermocoagulation is characterised by removing cervical lesions at a temperature of 100-120°C while cryotherapy freezes the cervix tissue (Naud et al. 2016; de Fouw et al. 2019). These treatment methods use portable devices that are affordable, safe, easy to use, quick, and do not require a highly skilled nurse (Maza et al. 2016). Cryotherapy and thermocoagulation have been evaluated to have similar performance and are effective at treating cervical cancer lesions (de Fouw et al. 2019; Naud et al. 2016). The cure rate of thermocoagulation has been reported to be9 1.4% for CIN1 and 91.6% for CIN2-3, whereas for cryotherapy it has been found to be 93.8% for CIN1 and 82.6% for CIN2-3 (de Fouw et al. 2019). A study done among a high risk population (HIV-positive women) reported that HPV test-and-treat was the most effective and cost-effective method and decreased the risk of cervical cancer by 56% (Campos et al. 2018). The incremental cost-effectiveness ratio was estimated to be $3010 USD per year of life saved (Campos et al. 2018).

1.7 HPV DNA testing on self-collected samples

1.7.1 Accuracy of self-sampling vs. clinician-sampling

Self-sampling is a desirable screening approach because it can motivate women to participate in the cervical cancer screening programme. It allows women to collect the specimens themselves in private, eliminates the time and financial costs of traveling to the clinics (Wong et al. 2016; Fargnoli et al. 2015). Women can perform self-collection at home or any private setting at their own time without experiencing the clinician's discomfort (Wong et al. 2016; Snijders et al. 2013). Previously, HPV testing was performed on cervical clinician-collected samples; however, it has evolved to vaginal self-collected sampling. Self-sampling allows samples to be collected from the vagina, containing a mixture of vaginal and cervical cells obtained using a cervicovaginal brush or swab (Pedersen et al. 2018). Self-collection was introduced to increase the screening coverage and eliminate the barriers of cytology testing

(Pedersen et al. 2018). In European countries, self-sampling for HPV tests is used as the alternative screening method for non-attendee women (Polman, de Haan, et al. 2019; Pedersen et al. 2018). Studies show a comparable detection rate of HR-HPV infection and clinical accuracy between self-collection and clinician-collection to identify CIN2 or higher (Nutthachote et al. 2019; Arbyn et al. 2014; Snijders et al. 2013). The agreement between self-collection and clinician-collection ranges from 0.60-0.88 (Nodjikouambaye et al. 2020).

Other studies have reported low self-collection performance compared to clinician-collection, attributed to different sampling devices or HPV test assays (Arbyn et al. 2014). A meta-analysis showed low sensitivity and specificity for self-collection compared to clinician-collection using a signal-based HPV testing assay (HC-2) (Arbyn et al. 2014). However, studies using clinically validated PCR-based HPV testing assays have demonstrated similar performance between self-collection and clinician-collection (Arbynet al. 2014). The pooled sensitivity of self-collection ranges from 77%-96% and 93%-96% for clinician-collection, while the specificity is 79%-84% for self-collection and 79%-86% for clinician-collection for detection of precancerous lesions (CIN2+) (Arbyn et al. 2018). Furthermore, self-collection has a better sensitivity than cytology tests. The incidence of CIN2+ among women with negative cytology tests was 3-fold higher than a negative self-collection test (Porras et al. 2015). Self-sampling provides access to cervical cancer screening for women from rural areas who cannot visit clinics or countries that do not have organised cervical cancer screening (Snijders et al. 2013). The self-collection strategy is suitable for the follow-up of women who test positive for HPV infection from low-income countries (Viviano et al. 2018). Self-collection provides the advantages of allowing samples to be mailed, stored, and reducing transportation costs (Arbyn et al. 2018). However, although self-sampling would be a good implementation in rural areas, women have raised some concerns about self-sampling such as fearing of collecting the specimen incorrectly. In additional, some potential barriers include loss to follow-up, and poor transport system (such as mailing of self-sampling) in rural areas (Mbatha et al. 2017; Mulki et al. 2021).

1.7.2 Experience and acceptability of self-sampling vs. clinician-sampling

Numerous studies have reported that a large proportion of women (61.9%-98.7%) are highly agreeable to self-collection and are willing to perform the screening tests in the future (Nodjikouambaye et al. 2019; Obiri-Yeboah et al. 2017; Sossauer et al. 2014; Bansil et al. 2014)**.** The self-sampling procedure has been demonstrated and has received high acceptability among women from indigenous communities and rural communities (Ogilvie et al. 2007; Zehbe et al. 2011; Racey et al. 2016; Bansil et al. 2014; Murchland et al. 2019). Studies highlight that self-collection is easy, more comfortable, less painful, and has understandable instructions to perform sampling (Nodjikouambaye et al. 2020; Maza et al. 2018; Bansil et al. 2014). The experience of self-sampling is associated with age and education. For example, women with less years of education (<13 years) were more likely to self-report less pain when performing self-sampling than clinician-sampling when compared to women with 13 years more years of education (Leinonen et al. 2018). Also, the experience of reporting fear for self-sampling was observed to decrease as age increases. A larger number of women younger less than 30 years were found to fear hurting themselves when performing self-sampling than those 30 years or more (Lorenzi et al. 2019).

The preference for self-collection ranges from 22.0%-95.0% compared to the preference for clinician-collection, which ranges from 11.9%-12.9% (Nelson et al. 2017; Polman, et al. 2019; Lorenzi et al. 2019). The higher preference for self-collection is due to the provision of privacy and the advantage of choosing the place and time to perform sampling (Lorenzi et al. 2019). Among African studies, preference for self-sampling for HPV testing ranges from 76.7%-86.3% (Haile et al. 2020). However, other studies reported that women prefer a clinician-collection due to the fear of collecting the specimen incorrectly or specimen inadequacy. Other factors include education and a lack of awareness for cervical cancer screening (Maza et al. 2018; Berner et al. 2013).

Since self-sampling is a feasible alternative for screening for cervical cancer in rural areas, different strategies have been designed to reach women who cannot visit the clinics or those who do not prefer the clinician to perform the sample collection (Jede et al. 2020; Arbyn et

al. 2018). These include, a direct offer of self-collection kits, mailing self-collection kits, and an opt-in strategy (Arbyn et al. 2018). Of these strategies, a community health worker's direct offer of self-collection had a high participation rate of more than 75% compared to other methods among underscreened women (Arbyn et al. 2018). Opt-in was observed to be a less effective strategy than mailing self-sampling kit/letter invitations for underscreened women (Arbyn et al. 2018). However, a recent randomised clinical trial of women with treated CIN2+ cases from the United States showed that the uptake of cervical screening increased with mailing HPV kits compared to the usual care reminder (26.3% vs 17.4%) (Winer et al. 2019).

1.7.3 Sampling devices and collection system

Sampling devices such as tampons, lavage, Evalyn brush, Qvintip, HerSwab, Catch-all swab, and Qiagen swabs have been used for the self-collection sample (Arbyn et al. 2018; Bishop et al. 2019). It has been shown that women favour swabs and brushes to collect a specimen for self-collection because they are easy to use, comfortable, and small in size (Bishop et al. 2019). Similarly, in a cross-sectional study performed by Bishop (2019) among women from the United States, the Catch-all swab device (73.9%), Qvintip (72.1%), and Evalyn (67.6%) were the most favourable sampling devices compared to HerSwab (49.4%) (Bishop et al. 2019). Furthermore, a study on a South African women cohort from rural regions (Mpumalanga) reported that cervical brushes were the most acceptable self-collection device (≥75%) compared to a tampon (19%) and lavage (4%) (Mahomed et al. 2014). However, in the urban areas (Johannesburg), women preferred tampon (45%) over lavage (33%) and cervical brush (22%) (Mahomed et al. 2014), indicating that women from different regions have different preferences for sampling devices.

These sampling devices have been evaluated in studies using clinically validated HPV testing assays. A brush or lavage device for self-collection provides a similar sensitivity to a clinician-collected sample with PCR-based HPV testing (Arbyn et al. 2014). In contrast, the signal amplification HPV testing has low sensitivity with any device used for sample collection (Arbynet al. 2014). The cervicovaginal lavage samples have been found to have significantly higher DNA concentrations than Qvintip samples (Jentschke et al. 2016). The clinical

performance for detection of CIN2+ was similar between the Evalyn (sensitivity of 89.8% and specificity of 66.7%) and Qvintip (sensitivity of 83.7% and specificity of 69.0%) sampling devices (Jentschke et al. 2016).

The sampling storage differs whereby swabs and Evalyn brush are stored dry, and brushes are placed in a liquid-based transport medium or smeared onto FTA cards (van Baars et al. 2012; Gonzalez et al. 2012; Gustavssonet al. 2009). Liquid transport medium is used as the standard storage system for HPV testing and has been shown to have comparable performance to dry storage or dry cervicovaginal sample collection (Sultana et al. 2015; Wolfrum et al. 2013). The dry specimen collection and liquid-based specimen collection were found to have an overall agreement of 92.8% with a kappa value of 0.85 to detect HPV infection (Sultana et al. 2015; Wolfrum et al. 2013). The positive agreement of HPV type-specific between dry and liquid-based sample collection was 91.3% for HPV16/18 with a sensitivity of 95.5% (Sultana et al. 2015). However, a liquid-based medium is not favourable for home-based self-collection kits as they are more likely to cause linkage and contamination without the nurse's supervision. Dry storage has a low cost for transportation and can be stored for an extended period at room temperature (Catarino et al. 2015; Gustavsson et al. 2009).

1.8 Risk factors for cervical cancer

1.8.1 Sexually transmitted pathogens associated with HPV and development of cervical cancer

Since HPV is sexually transmitted, other STIs share the same transmission route, thus the risk factors that influence the acquisition of other STIs and the development of cervical cancer are equivalent. STIs are believed to increase the acquisition and spread of each other through sexual behaviour and biological mechanisms such as altering pathogens' natural history, interfering local immune response and disruption of mucus (mucosal) (Moscicki et al. 2010; Wright 2009; Horvath et al. 2010; Castle and Giuliano 2003). Furthermore, STIs have been found to function as cofactors of HPV infection, and co-infection increases the inflammation, resulting in oxidative metabolites and reduction of cell-mediated immunity (Verteramo et al. 2009; Samoff et al. 2005; Castle and Giuliano 2003). Therefore, STIs have been reported to increase the acquisition of HPV infection and persistent HPV infection, thereby increasing the risk of cervical cancer (Samoff et al. 2005; Wang et al. 2011).

1.8.1.1 Sexually transmitted infections

Sexually transmitted infections (STIs) such as *Chlamydia trachomatis* (*CT*), *Trichomonas vaginalis (TV)* and *Neisseria gonorrhoeae (NG)* are linked with inflammation, leading to susceptibility to infection with HPV and growth of cancerous cervical cells (Castle and Giuliano 2003; Reighard et al. 2011; Castle et al. 2001; Silva, Cerqueira, and Medeiros 2014). Cancer is associated with inflammation, and different pathways trigger inflammation to cause malignancy. These include an increase of cytokines and chemokines production, which are the key components for cancer-related inflammation (Germano, Allavena, and Mantovani 2008; Costantini et al. 2009; Moerman-Herzog and Nakagawa 2015). These inflammatory mediators (cytokine and chemokines) can suppress the HPV viral gene expression, thereby forming an inflammatory microenvironment that can result in cell damage and encourage cellular to change, resulting in cancer (Moerman-Herzog and Nakagawa 2015; Mantovani et al. 2008). Also, oncogenic genes (HPV16 E5, E6 and E7) can increase the inflammatory cyclooxygenase-prostaglandin (COX) axis through overexpression of receptors for E-series prostaglandin and overexpression of oncogene COX-2, which can encourage immune infiltration, inflammation, and cancer progression (Adefuye and Sales 2012; Oh et al. 2009; Subbaramaiah and Dannenberg 2007; Oh et al. 2010).

The co-infection of sexually transmitted pathogens is thought to influence CIN's progression through mucosal immunity, which may result in persistent HPV infection and cervical cancer lesions (Kawana et al. 2008; Chumduri et al. 2013; Kojima et al. 2011; Kiseki et al. 2017). Infection with *TV* increases the likelihood of cervical abnormalities in women with HR-HPV infection (Donders et al. 2013), which is a result of induced inflammation that causes disruption in the cervical epithelium, may allow entry of HPV infection and interferes with viral clearance (Thurman and Doncel 2011; da Costa et al. 2005). A study recognized *NG* and *CT* as primary STIs related to HR-HPV infection for elevated risk of all cervical abnormalities with the implication that an interaction may occur in the development of cervical cancer lesions (de Abreu et al. 2016). A longitudinal study conducted by Lehtinen and co-authors showed that CT was an independent risk factor for CIN2 (Lehtinen et al. 2011). The authors suggest that this STI may be involved in the early development of the precancerous lesion but

it was not observed in women with high grade lesions (CIN3) (Lehtinen et al. 2011). Di Pietro et al., 2018 reported that women co-infected with HPV and CT were more likely to have highly diverse cervical microbiota than women negative for HPV/CT confection, thus increasing their susceptibility to persistent infection (Di Pietro et al. 2018).

1.8.1.2 Herpes simplex virus type-2

Herpes simplex virus (HSV) type 2 is the most common HSV serotype and has been found to have a synergistic function with HPV infection in causing cervical cancer. Mechanisms of HSV-2 involve in causing precancerous lesion or cancer include mutations, inflammation, and formation of ulcer (Konvalina, Gašperík, and Golais 2002; Clure and Rivard 2018; Tomkins, White, and Higgins 2015). These mechanisms may cause genetic changes and damages the epithelium barrier thereby allowing the entry of HPV into the epithelial cells and the growth of cervical lesions (Horbul et al. 2011; Clure and Rivard 2018; Konvalina, Gašperík, and Golais 2002). Infection of HSV-2 engages in the first stage of carcinogenesis ("hit and run" mechanism). Therefore, retention of HSV-2 is not needed for later stages in cancer progression. Hence, infection of this virus is not consistently found in all biopsies of precancerous lesions or cancer. This indicates that HSV-2 infection is required for the initial cell transformation but not the development of cancerous lesions/cancer (Zhao et al. 2012; Galloway and McDougall 1983; Partridge et al. 2007; Smith, Herrero, et al. 2002). *In vitro* studies showed that the transcription level of HPV genes (E1, E2 and E6) increased by 3-fold in CaSki cells and 9-fold HeLa cells with HSV-2 infection, indicating that HSV-2 might increase cervical cancer risk by the induction of overexpression of the HPV regulatory and oncogenes (Pisani et al. 2002; Pisani et al. 2004). HSV-2 is also associated with HIV acquisition and persistent HPV infection, which might be due to the shared behavioural risks including route of transmission (Caldeira et al. 2013). Li and Wen (2017) showed that HSV-2 seropositive women were 2-times more likely to have high-risk of cervical cancer than seronegative women and after adjusted for HR-HPV infection, indicating that being positive for HSV-2 might act as an independent risk factor for cervical cancer (Li and Wen 2017). Significantly increased co-infection of HSV-2 with HPV infection was observed in CIN and squamous cell carcinoma cases compared to having single HSV-2 or HPV infection (Zhao et al. 2012).Therefore,

demonstrating that HSV-2 or coinfection of HPV/HSV-2 may impact the progression of cervical cancer (Zhao et al. 2012).

1.8.1.3 HIV infection

Genital HPV infection is significantly associated with HIV acquisition and the interaction between these viral STIs is complex (Williamson 2015). Both these viruses (HPV and HIV) share the same transmission route and remain the major public health problem in sub-Saharan Africa (Mbulawa et al. 2013). HIV-positive women have compromised immune systems, which is a favourable environment for persistent HPV infection and the development of premalignant lesions (Lissouba, Van de Perre, and Auvert 2013). Globally, it is estimated that 10% of HIV-positive women are likely to develop CIN2-3 compared to 1-2% of HIV-negative women yearly (Arbyn et al. 2012; De Vuyst et al. 2012; Joshi et al. 2013; Denny et al. 2008; Zhang et al. 2012). HIV-positive women have a relatively higher risk of acquiring HPV infection and a lower rate of clearing the infection than HIV-negative women (Liu et al. 2018). A cohort study among women with HIV-1 infection from Zimbabwe showed that HPV clearance was negatively associated with HIV-1 infection which shows that immune response may play a role on the increase of HIV-1 susceptibility (Averbach et al. 2010). Coinfection of HIV infection is significantly associated with genital HPV prevalence and HPV type specific concordance (Mbulawa et al. 2009). HIV-positive women have been reported to have increased prevalence of HPV infection and are more likely to be infected with multiple HPV genotypes than HIV-negative women. In a heterosexual couple South African study, HPV prevalence was significantly higher among HIV-positive women/men compared to HIV-negative women/men (Mbulawa et al. 2010). The study also found that male participants having a HIV-positive partner had higher risk of having HR-HPV infection and LR-HPV infection than male participants with HIV-negative partners (Mbulawa et al. 2010)

The influence of high HPV acquisition rate and HPV prevalence were associated with reduced CD4 cell count (<350 cells/mm^3) (Liu et al. 2018; Mbulawa et al. 2013). Furthermore, low CD4 count was significantly associated with almost 3-fold increased risk of developing HSIL, indicating a poor immune response among HIV-positive women (Enebe et al. 2015). Studies

of prospective cohorts report that HIV-positive women on antiretroviral therapy (ART) had reduced progression of SIL, increased clearance of HR-HPV types(16/18) and low incidence of abnormal cytology than women not on ART (Blitz et al. 2013; Adler et al. 2012). Therefore, this indicates that long-term exposure to ART plays a significant role in improving HIV-positive women's immunity and may protect against cervical lesions' progression (Kelly et al. 2018; Zeier et al. 2015). It has been found that reduced HIV viral load among women on ART for more than two years increased the likelihood of HPV clearance (Konopnicki et al. 2013).

1.8.1.4 Pathobionts

Pathobionts are microbes that live in a non-harmful symbiosis that can potentially influence or cause clinical disease in healthy humans without showing any noticeable symptoms (Pickard et al. 2017; Hornef 2015; Jochum and Stecher 2020; Chow, Tang, and Mazmanian 2011). These microbes are also known as opportunistic pathogens that reside as commensals in the healthy environment and can increase chronic inflammatory responses in a genitally vulnerable host (Chow, Tang, and Mazmanian 2011; Combaz-Söhnchen and Kuhn 2017; Bjartling, Osser, and Persson 2010; Zechner 2017). *Mycoplasma hominis (MH)*, *Mycoplasma genitalium (MG)*, *Ureaplasma spp* (*U. urealyticum*, and *U*. *parvum*) belong to a genus of genital mycoplasma from the *Mycoplasmataceae* family (Martin 2015; Taylor-Robinson 2017). These microorganisms have been isolated in 67% of sexually active, 40% sexually inactive, and 25% of postmenopausal women (Viscardi 2010). It has been reported that *MH* was present in 20-50%, *MG* in 0-5% and *Ureaplasma* spp in 40-80% of sexually active women from vaginal/cervical samples (Morse 2010). Studies show inconclusive results on whether these microorganisms are potential genital pathogens or commensal inhabitants (Combaz-Söhnchen and Kuhn 2017; Kletzel et al. 2018). However, genital *mycoplasma* is associated with cervicitis, vaginitis, infertility, discharge, and pelvic inflammatory disease (Combaz-Söhnchen and Kuhn 2017; Leli et al. 2018; McGowin, Popov, and Pyles 2009).

Notably, emerging evidence show that genital *mycoplasma* might impact HPV natural history through initiating viral persistent infection and cellular abnormalities (Ye et al. 2018; Lukic et al. 2006). *U. urealyticum has been established as the co-factors of HPV infection (prevalence)*

and may influence persistent disease and cervical abnormalities (Lukic et al. 2006). The bacterial load of *U. parvum* in women with cervical lesions was significantly higher than those with no lesions (Amorim et al. 2017). Women with co-infection of *U. urealyticum* and HR-HPV infection had a 12-times higher risk of HSIL and cervical cancer (Wang et al. 2019). The risk of HSIL and cervical cancer was 11.6-fold higher for *U. urealyticum* and 7.5-fold higher for *U. parvum*, demonstrating that the presence of *Ureaplasma* species might be biomarkers for cervical cancer lesions (Wang et al. 2019). The pathogenesis mechanisms of *Ureaplasma* species are reported to be unclear. However, *MH* was found to increase inflammatory cytokine expression from the monocytic cell line, thus increasing the risk of cervical abnormalities (Fiori et al. 2013).

1.8.2 Other factors

1.8.2.1 HPV Viral load, HR-HPV types, and persistent infection

The viral load of HPV infection is regarded as a biomarker for progression of cervical lesion to cancer and increases with higher grades of cervical neoplasia (Malagón et al. 2019; Marongiu et al. 2014; Kim et al. 2020). An increase in HPV viral load over time was associated with a 4-fold increase in prediction of the development of CIN2-3 (Mittal et al. 2017). According to Malagon et al., 2019, the relationship between high-grade cervical lesions and viral load was due to lifestyle aspects (such as contraceptive use) that affect immunity (Malagón et al. 2019). Cross sectional studies found a significant association of high HPV viral load with an elevated risk of having a large size of the cervical lesion and a higher grade of squamous lesions (Sun et al. 2001; Ding et al. 2016; Kim et al. 2020; Wang et al. 2018). However, having low viral load was not associated with an increased risk of squamous intraepithelial lesions and cervical lesion size (Sun et al. 2001; Ding et al. 2016), therefore indicating that the progression from HPV infection to HSIL is driven by high HPV viral load and that viral load could be utilised to estimate the cervical lesions' severity.

Previous studies have shown that high viral load infections have reduced clearance rates and increased persistent infection likelihood, suggesting that high viral load could indicate persistent infection (Mittal et al. 2017; Trevisan et al. 2013). Persistent infection with HPV16, 18, 31, and 33 may influence the probability of developing high-grade cervical lesions (Kjær et al. 2010). A prospective study done by Matsumoto (2011) demonstrated a significantly

higher cumulative incidence rate of CIN3 for women with HR-HPV specific types (16, 18, 31,33, 35, 52, and 58) compared to women with other high-HPV types (20.5% vs.6.0%) within five years of follow-up (Matsumoto et al. 2011). Also, HPV16/18 has been discovered as a strong predictor of the increased risk of squamous cell carcinoma and adenocarcinoma (Arnheim Dahlström et al. 2011). Therefore, this shows that these are essential HR-HPV types that play a significant role in development of invasive cervical cancer. Other studies show that women with cytology abnormalities (ASCUS, LSIL and HSIL) are more likely to have multiple HPV infections than those with normal cytology (Schmitt et al. 2013). The presence of multiple HR-HPV infections with high viral load was found to have 4-6 fold increased risk of developing cervical precancerous lesions than with single infection and high viral load (Schmitt et al. 2013).

1.8.2.2 Smoking

Smoking is associated with HR-HPV infection, and tobacco or cigarette smoke contains a chemical (such as Nicotine) that causes damage to the squamous epithelial cells, promoting the growth of cervical cancer lesions (Castle 2008; Fang et al. 2018; Wang et al. 2017; Chen and Wang 2019; Nersesyan et al. 2020; Alam et al. 2008; Jensen et al. 2012a). Two mechanisms have been reported to influence cervical lesions' development, namely the inhibition of immune response by tobacco metabolites and exposure of cells infected by HR-HPV infection, which causes damage to the DNA (Castle 2008; Castellsagué and Muñoz 2003). A study done by Xu (2018) reported that women currently smoking had a 43% increased risk of high-grade CIN compared to women who never smoked (Xu et al. 2018). Current or past smokers were observed to have an almost 4-times higher risk of CIN2 incidence than non-smokers (Sarian et al. 2009). The odds of developing cervical cancer significantly increased with long-term smoking and in-take/dose of cigarettes per day (Sugawara et al. 2019; Jensen et al. 2012b). Similarly, women with persistent HPV infection and an intake of 18 or more cigarettes per day were found to have a higher risk of CIN3+ than those who never smoked (Fang et al. 2018). Moreover, a prospective study conducted by Jensen (2012) reported that women having persistent high-risk infections with ≥ ten years of smoking and an intake of 20 or more cigarettes per day were more likely to be diagnosed with high-grade CIN3+ within 13 years of follow-up (Jensen et al. 2012b).

1.8.2.3 Sexual behaviour and parity

Sexual behaviour is associated with HPV infection and increases the progression to cervical cancer (Louie et al. 2009; Itarat et al. 2019). Women who have a young age of sexual debut have been consistently found to have a high risk of HPV infection (Louie et al. 2009). Women who had their first sexual experience before the age of 15 years had an almost 6-times higher risk of developing precancerous lesions (Kassa 2018). Having multiple sexual partners (≥5) was correlated with a six-fold increased risk of precancerous lesions (Kassa 2018). Furthermore, the risk increases with age difference whereby women having an age difference of 10 years or more with their sexual partners had a 2-fold increased risk of developing cervical cancer (Baussano et al. 2017).

Parity is related to sexual behaviour and has been found to increase cervical cancer risk. The association between parity and cervical cancer progression is suspected to be influenced by the elevated levels of sex hormones during pregnancy (Roura et al. 2016; Rajkumar et al. 2006; Muñoz et al. 2002). During pregnancy, it has been found that changes in the squamous columnar junction occur, which may enable HPV infection, influence persistent infection, and accelerate the development of cervical lesions and cervical cancer (Rajkumar et al. 2006; Jensen et al. 2013; Muñoz et al. 2002). Another biological mechanism is the immune suppression triggered by high progesterone levels, which may increase HPV acquisition and development of cervical lesions (Gariglio et al. 2009; Delvenne et al. 2007; Rajkumar et al. 2006). A study done among women with persistent HPV infection reported that having more than two pregnancies resulted in an almost 2-times higher risk of CIN3+ within 13 years of follow-up (Jensen et al. 2013). Similarly, women with more than five children were nearly 3-times more likely to have high cervical cancer risk (Paramita 2010).

1.8.2.4 Contraceptive use

The use of hormonal contraceptives is reported to regulate HPV immune responses and encourage the risk of persistent infection or cervical lesion progression (Volpato et al. 2018; Marks et al. 2011). Women currently using hormonal contraceptives were observed to have a 50% higher risk for cervical lesions progression than former users or women who never used

hormonal contraceptives (Xu et al. 2018). A case-control study among healthy women and cervical cancer confirmed women found that current users or women who had ever used oral contraceptives were more likely to have increased risk of cervical cancer than women who never used oral contraceptives (Vanakankovit and Taneepanichskul 2008). Furthermore, in a case-control study among black South women, prolonged use injectable contraceptives but not oral contraceptives was significantly correlated with a transiently higher risk of cervical cancer but not in women that had ceased the use more than ten years previously (Urban et al. 2012). Similarly, women with long-term oral contraception (≥ 15 years) had a significantly higher risk of high-grade cervical lesions, carcinoma in situ and invasive cervical cancer; indicating that long term use of oral contraceptive might be a cofactor of cervical cancer (Roura et al. 2016; Xu et al. 2018; Vanakankovit and Taneepanichskul 2008). However, other controlled studies have shown that women who using either oral or injectable contraceptives only or combined showed no significant increased risk of cervical cancer (Shapiro et al. 2003; Peng et al. 2017). Therefore, this difference could be affected by some uncontrolled risk factors (such as sexual behaviour) that may have a negative impact on the results.

1.9 Project motivation

The prevalence of HPV and cervical cancer is high in South Africa and is further exacerbated by the HIV epidemic (Bruni et al. 2019a; Stein et al. 2008; Mbulawa, Coetzee, and Williamson 2015; Mbulawa et al. 2009; Mbulawa et al. 2010). The Eastern Cape Province has a high ASR of cervical cancer (29.2 per 100 000) (Somdyala et al. 2015b; Somdyala et al. 2020) and some of the poorest health infrastructure. These women are informed about cervical cancer screening when the visit clinics. Women in rural areas such as Eastern Cape are not likely to be screened for cervical cancer because of the lack of knowledge, awareness regarding cervical cancer screening, health facilities and resources to perform screening (Ramathuba et al. 2016; Godfrey, Mathenjwa, and Mayat 2019; Sibiya and Grainger 2007). The high incidence rate of cervical cancer in the Eastern Cape stresses the need to improve cervical screening, particularly in rural areas. The introduction of self-collection of samples could increase the number of women accessing cervical cancer screening, as this is an easy and accessible screening method. Not much research has been done in Eastern Cape on the prevalence of HPV and typing of HPV as well as the acceptability of self-sampling as part of a cervical screening program. Therefore, the findings of this study will add to the limited information

on HPV and cervical cancer screening. Furthermore, this study will raise awareness of the risk factors associated with HPV types causing cervical cancer. It will also compare the performance of two HPV diagnostic tests namely HC2 and real-time *hpVIR* for HPV detection among women from the rural community clinic and referral clinic. The HPV typing data will provide background information to support the ongoing HPV vaccination programs. Currently, there is limited knowledge on the prevalence of STIs and high-risk HPV types causing cervical cancer. Further research questions will be identified based on the data generated during the study.

Therefore, the objectives of the study are:

- To investigate the prevalence of HR-HPV and factors associated with HR-HPV infection among women from rural Eastern Cape, South Africa.
- To investigate the distribution of HPV genotypes among women with cervical intraepithelial lesions according to HIV status from Eastern Cape Province, South Africa.
- To investigate HR-HPV prevalence and compare agreement between clinician-collected and self-collected genital specimens as well as two different HPV tests on clinician-collected samples.
- To investigate the prevalence of sexually transmitted pathogens and co-infection with HR-HPV infection among women from rural Eastern Cape Province, South Africa.

CHAPTER 2: Human papillomavirus prevalence and risk factors among HIV-negative and HIV-positive women residing in rural Eastern Cape, South Africa

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ABSTRACT

Background: This cross-sectional study investigate the prevalence of high-risk (HR) human papillomavirus (HPV) and factors associated with HR-HPV infection among women from rural Eastern Cape, South Africa.

Method: HPV prevalence was determined using the Hybrid Capture-2 assay in cervical specimens from 417 women aged ≥30 years (median: 46 years, range: 30-98 years) recruited from the community health clinic in the Eastern Cape.

Results: HR-HPV prevalence was 28.5% (119/417), and HIV-positive women had significantly higher HR-HPV prevalence than HIV-negative women (40.6%, 63/155 vs 21.4%, 56/262 respectively, *p*=0.001). HIV-positive status (OR:: 2.52, 95% CI: 1.63-3.90); having ≥3 lifetime sexual partners (OR: 2.12, 95% CI: 1.16-3.89); ≥1 sexual partner in the last month (OR: 1.89, 95% CI: 1.21-2.92); ≥4 times frequency of vaginal sex in the past 1-month (OR: 2.40, 95% CI: 1.32-4.35) and having vaginal discharge currently/previous week (OR: 2.13, 95% CI: 1.18-3.85) increased the risk of HR-HPV infection. In multivariate analysis, HIV-positivity remained strongly associated with HR-HPV infection (OR: 1.94, 95% CI: 1.17-3.22).

Conclusion: Risk factors related to sexual behaviours play a significant role in HR-HPV infection in this population. This report will inform health policy makers on HPV prevalence in this population and contribute to discussions on use of HPV testing as primary cervical screening test in the South Africa.

2.1 INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted virus (Muñoz et al. 2003). Cervical persistent infection with any of the high-risk (HR) HPV genotypes; such as HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and/ or 68; is associated with development of cervical intraepithelial lesions and cancer (Bouvard et al. 2009). There are a number of factors influencing HPV natural history (i.e., HPV acquisition, clearance, persistence and transmission) and development of cervical intraepithelial lesions and cancer (Castellsagué et al. 2014; Schiffman and Kjaer 2003; Strickler et al. 2005).

The effect of HPV and HIV-co-infection has been established, and women co-infected with both HIV and HPV are found to have reduced HPV clearance rate as well as an increased risk of persistent HPV infection compared with HIV-negative women (Strickler et al. 2005; Rowhani-Rahbar et al. 2007). HIV-positive women have a significantly increased risk of developing cervical lesions as they are more likely to have persistent HR-HPV infection (Hawes et al. 2003). Moreover, studies have found higher HR-HPV prevalence, HR-HPV viral load and cervical lesions among women who are HIV-positive compared to HIV-negative (Clifford et al. 2016; Mbulawa et al. 2014). South Africa has a high burden of HIV infection; in year 2018, 20.4% women between the ages of 10 and 49 years were reported to be living with HIV (UNAIDS 2019).

Globally, the cervical cancer incidence (13.1 per 100,000 women) and mortality rate (6.9 per 100,000 women) are high among women of reproductive age (15-44 years) (Bruni et al. 2019b; Fitzmaurice et al. 2017). Africa has the highest burden of both HPV and cervical cancer (de Martel et al. 2012; Ogembo et al. 2015). Of the reported HPV prevalence globally (11.7%) (Bruni et al. 2010), the estimated overall prevalence is two-fold higher (29%) among African women with normal cervical cytology (Ogembo et al. 2015). HPV prevalence has been found to differ across Southern African regions (Ogembo et al. 2015). This is also observed in South African studies, with prevalence ranging between 20.4% to 76% among women with normal cervical cytology (Allan et al. 2008; Ebrahim et al. 2016; Giuliano et al. 2015; McDonald et al. 2012; Richter et al. 2013). The different HPV prevalence in these studies could be influenced

by many factors including different HPV detection assay, type of specimen used, the age of the study population, HIV co-infection and study population characteristics.

HPV vaccination programs are implemented in number of countries including South Africa as the primary tool for the prevention of HPV infection and HPV-associated diseases; however, cervical cancer screening is essential for both the vaccinated and unvaccinated populations (Denny and Kuhn 2017). The implementation of an effective cervical cancer screening program that offers regular cervical screening, monitoring, follow-up, and management of cervical lesions is needed in order to prevent or manage the progression to invasive cancer, especially in a country where the HIV burden is high, like South Africa (Kabir et al. 2012; Tartaglia et al. 2017). The burden of cervical cancer has significantly decreased in developed countries due to the success of cervical screening implementation (Bruni et al. 2010; Jemal, Ward, and Thun 2010). However, in African regions, especially in rural areas, cervical screening remains a major issue because of limited resources, lack of knowledge or awareness and lack of trained health care professionals (Arulogun and Maxwell 2012; Perlman et al. 2014)

Currently, in South Africa, the Pap smear/cervical cytology remains the only screening test utilised for cervical cancer screening in public facilities. The targeted age group for cervical cytology in the general population is women aged 30 years or older, and the recommendation is to have cervical screening at least three times in their lifetime (Botha and Dreyer 2017). However, among HIV-positive women, cervical cancer screening begins at the aged <30 years or at HIV diagnosis, and the policy recommends 3-year intervals if the cytology result is negative or annual screening if the cytology result is positive (National Department of Health 2017). Cervical cancer screening coverage is lower in rural areas, which are generally poor and lack the health care services found in urban areas. Data from the South African National Health Laboratory Services (NHLS) indicate that among HIV-positive and HIV-negative women age ≥30 years, Pap smear coverage in Eastern Cape municipality districts was <50% during 2013-2014. This is well below the national target of 70% Pap smear coverage (Makura et al. 2016).

In many countries, cervical cytology is being replaced by HPV testing in cervical screening programs. (Altobelli et al. 2019; Basu et al. 2018). There is increasing evidence showing that HPV testing identifies more cases of precancerous cervical changes when compared to cytology screening (Dillner 2019; Ogilvie et al. 2018). A randomised trial study performed in Netherlands reported a higher detection rate of women with CIN2+ after HPV testing (16.5 per 1,000 women) when compared to cytology testing (10.1 per 1,000 women) at baseline (Ogilvie et al. 2018). However, the detection rate of women with CIN2+ was significantly lower with HPV testing (5.0 per 1,000 women) than cytology testing (10.6 per 1,000 women) after a follow-up of 48 months (Ogilvie et al. 2018). Since HPV testing has been established to be more highly sensitive than cytology testing, the cervical screening interval of women with an HPV-negative test have been extended from 5 to 10 years because of low long-term risk of CIN3+ development (Dillner 2019; Peto and Gilham 2017). There are different scenarios to manage HR-HPV-positive women. In low-resource settings, screen-and-treat regimens have been investigated for managing HPV-positive women (Kuhn and Denny 2017). In recent years there has been discussion of modifying the South African guidelines to include algorithms with HPV testing and strategies of treating HR-HPV-positive. There is now good evidence that HPV testing could replace or complement cytology (Botha and Dreyer 2017; National Department of Health 2017).

According to the Eastern Cape Province population-based cancer registry, cervical cancer became the most common cancer between 1998 and 2012, with a significant increase in incidence during the period from 22.1 per 100,00 to 29.0 per 100,000 (Somdyala et al. 2015a). However, there is a lack of information on HPV prevalence and high-risk types causing cervical cancer among women residing in rural Eastern Cape Province of South Africa. It is crucial to investigate the prevalence of HPV, as this information will serve as baseline data to identify the need and appropriate strategies of prevention and cervical screening implementation, since there is high burden of cervical cancer in this region. Therefore, the aim of this study was to investigate the prevalence of HR-HPV and the association of socio-demographic data, sexual behaviour, and other factors with HR-HPV among women aged 30 years or older, in the general population who were visiting a community-based clinic in rural Eastern Cape Province.

2.2 MATERIALS AND METHODS

2.2.1 Study design and specimen collection

This cross-sectional study was carried out at a community health clinic within OR Tambo district municipality, in Mthatha, Eastern Cape Province of South Africa. A total of 417 women, aged 30-98 years who were attending the community health clinic for cervical cancer screening and for any other reasons were recruited between September 2017 and August 2018. The study adhered to the Helsinki declaration of 2013. Written informed consent was sought from prospective participants. The Human Research Ethics Committees of the University of Cape Town (UCT) (HREC reference 615/2017), Walter Sisulu University (reference 090/2016) and Eastern Cape Department of Health Ethics (EC reference 2017RP0_484), approved all aspects of the study. Sociodemographic data and information on risk factors were obtained through interviews.

The study nurse collected two cervical specimens for cervical cytology and HPV testing. Cervical brushes for HPV testing were stored in Digene transport medium (Qiagen, Gaithersburg, MD, USA) and transported to UCT HPV laboratory where they were stored at -80^{o}C until laboratory testing. “The NHLS laboratory, Mthatha, performed the cervical cytology analysis and results were interpreted according to the Bethesda Classification System (Solomon et al. 2002).” All women who had high-grade squamous intraepithelial lesions (HSILs) on their cytology result were referred to Nelson Mandela Academic Hospital colposcopy clinic for follow-up.

2.2.2 HPV DNA detection and viral load

The Hybrid Capture-2 (HC-2) DNA test (Qiagen Inc., Gaithersburg, MD; USA) identifies 13 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68); this test was performed manually according to manufacturer’s instructions. Viral quantification data in ratio of relative light units/cut off (RLU/CO) was measured using a DML 3000^{TM} microplate Luminometer (Qiagen Inc., Gaithersburg, MD; USA); a specimen with a ratio of ≥1 (5,000 copies) was considered a positive result and a specimen with ratio < 1 was considered a negative result for HR-HPV types. The RLU reflect the viral load but in this test there is no discrimination on HPV type.

2.2.3 Statistical analysis

Data were collected in REDcap (Research Electronic Data Capture) and the statistical analysis performed using Stata 14.2 (Stata Corp, College Station, Texas). Most of the data were categorical. When data were presented as proportions of the total sample, the missing data were excluded from the denominator. Univariate logistic regression models were used to identify the factors associated with HR-HPV. Multivariate models included factors that were statistically significant at 5%, considering variables with *p*-value of less than 10% in the univariate analysis. The figure was drawn using GraphPad Prism v6.01 (GraphPad Software, Inc., San Diego, CA); this summarises the HPV viral load disaggregated by HIV status and cervical cytology. The Mann-Whitney test was used to determine differences in median HR-HPV viral load between HIV-positive and HIV-negative women with normal or abnormal cytology and a p-value of <0.05 was considered significant.

2.3 RESULTS

2.3.1 Description of study participants

Of the 417 participants, 37.2% (155/417) were HIV-positive, and 96.1% of the HIV-positive women were on antiretroviral drugs. The median age of the study participants was 46 years (interquartile range (IQR) 38-55 years), with the majority in the age group ≥50 years (42.9%). The median age at first sex was 18 years (IQR 16-20 years), and the median lifetime number of sexual partners was 2 (IQR 2-3). The majority of women had never smoked (94.5%), never drank alcohol (90.8%), and had been pregnant before (96.6%). The highest education level attained by most of the study participants was high school or university (73.4%) and they had a low monthly household income<$139.36 (71.2%). Amongst the 154 (36.9%) women who reported using contraception with their current partners, 79.2% (122/154) had started using contraceptives at the age of 18 years (Table 2.1). According to their genital health history, 53.7% (224/417) of women self-reported having had a vaginal discharge and 14.4% (60/417) as having had ulcers/blisters/warts in their lifetime. A pelvic examination by the study nurse revealed that on the day of specimen collection, a vaginal discharge was present in 20.9% (87/417) and warts in 1.2% (5/417) of the women.

2.3.2 HR-HPV prevalence and HIV-coinfection

The overall HR-HPV prevalence was 28.5%. HIV-positive women were found to have higher HR-HPV prevalence compared to HIV-negative women (40.6%, 63/155 vs 21.4%, 56/262, respectively; *p*<0.001, Table 2.1). HIV-positive women had significantly higher HR-HPV viral load as measured by RLU compared to HIV-negative women [median: 0.390 (0.009-3245.33) compared to 0.180 (0.08-2884.2) RLU/CO, *p*<0.0001, Figure 2.1] and this remained so even when women were grouped according to cervical cytology results [normal: 0.270 (0.009-1557.42) RLU/CO vs 0.180 (0.08-1660.34) RLU/CO, p<0.0001 and abnormal: 75.350 (0.08-3245.33) vs 0.200 (0.09-2884.20) RLU/CO, *p*=0.035, Figure 2.1].

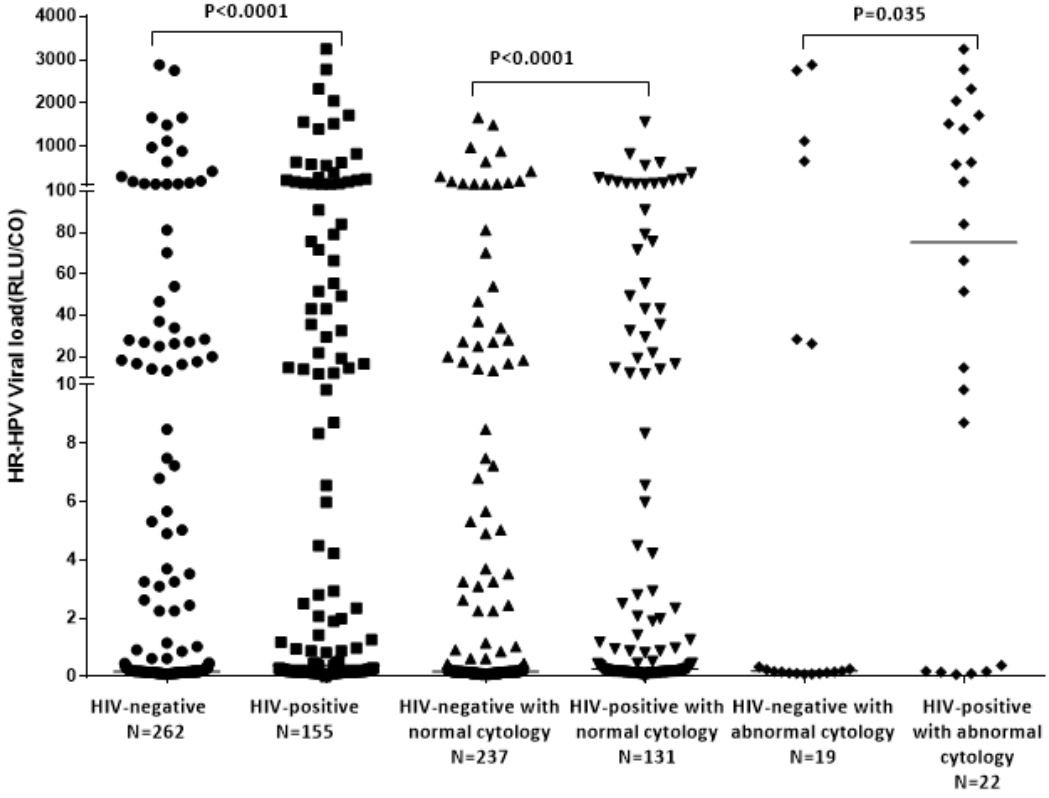

Figure 2.1: High-risk human papillomavirus (HR-HPV) viral load according to human immunodeficiency virus (HIV) status and cervical cytology

2.3.3 Factors associated with HR-HPV in women (univariate and multivariate analysis)

Women with more than three lifetime sexual partners [odds ratio (OR), 2.12 (95% confidence interval (CI):1.16-3.89)], one or more sexual partners in the last month [OR: 1.89 (95% CI:1.21-2.92)] and currently using any contraception [OR: 1.86 (95% CI:1.02-2.87)] were found to have significant increased risk of HR-HPV. Furthermore, women who reported to have had vaginal sex ≥4 times during the past 1 month were more likely to have HR-HPV [OR: 2.40 (95% CI:1.32-

4.35)] compared to those who reported no sexual intercourse during past 1-month (Table 2.1). Risk of HR-HPV was also found to increase with increasing number of sexual partners in the past 12-months compared to those who reported no sexual partner in the past 12-month [1 partner: OR: 1.99 (95% CI:0.35-1.40) and ≥2 partners: OR: 2.17 (95% CI:0.97-4.84)], however this was not statistically significant for women with ≥2 sexual partners in the past 12months.

Participants who self-reported to having vaginal discharge at the time of the study (currently) or recent (in the last week at the time of the study) had increased risk of HR-HPV types [OR: 2.13 (95% CI:1.18-3.85) when compared to women who reported not having had a vaginal discharge in their lifetime. It is important to note that during pelvic examination by the study nurse, all women who reported having a current discharge or discharge in the previous week before the interview or examination, were found to have a discharge on the day of the study visit. Even though self-reported warts history was not associated with HR-HPV, 60.0% (3/5) of women who were found to have warts during the pelvic examination were also found to have HR-HPV infections. Other factors such as age at first sex, level of education, annual household income, smoking, self-reported ulcer or warts and pregnancy were not significantly associated with HR-HPV infection (Table 2.1). In the multivariate analysis, HIV-positive status [OR: 1.94 (95% CI: 1.17-3.22)] and abnormal cytology [OR: 2.83 (95% CI: 1.39-5.76] were associated with an increased risk of HR-HPV types. Women who reported having new sexual partners in the last 1-month had increased risk of HR-HPV types; however, this was not statistically significant [OR: 1.80 (95% CI: 0.68-4.97)] (Table 2.1).

Table 2. 1: Factors associated with high-risk human papillomavirus in women (univariate analysis and multivariate analysis).

		HR-HPV prevalence	Univariate analysis		Multivariate analysis	
Variables	n/N (%)	n (%)	OR (95% CI)	p-value	OR (95% CI)	*p*-value
Age in years: median (IQR)	46 (38-55)	119/417 (28.5)	0.97 (0.95-0.99)	**0.001**	0.99 (0.96-1.02)	0.49
HIV Status						
Negative	262/417 (62.8)	56/262 (21.4)	ref		ref	
Positive	155/417 (31.2)	63/155 (40.7)	2.52 (1.63-3.90)	**<0.001**	1.94 (1.17-3.22)	**0.01**
Age categories						
30-39 years	130/417 (31.2)	46/130 (35.4)	ref			
40-49 years	108/417 (25.9)	40/108 (37.0)	1.07 (0.63-1.83)	0.79		
≥50 years	179/417 (42.9)	33/179 (18.4)	0.41 (0.25-0.70)	**0.001**		
Highest level of education attained						
Never/ Primary	111/417 (26.6)	25/111 (22.5)	ref			
High School/University	306/417 (73.4)	94/306 (30.7)	1.53 (0.92-2.53)	0.1		
Household income						
<$139.36	297/417 (71.2)	82/297 (27.6)	ref			
≥$139.36	115/417 (27.6)	36/115 (31.3)	1.19 (0.75-1.91)	0.46		
Smoking status						
Never	394/417 (94.50)	110/394 (27.9)	ref			
Former smoker/Current smoker	23/417 (5.5)	9/23 (39.1)	1.66 (0.70-3.95)	0.25		
Ever drank alcohol						
No	379/417 (90.8)	111/379 (29.3)	ref			
Yes	38/417 (9.1)	8/38 (21.1)	0.64 (0.29-1.45)	0.29		
Age at first sexual intercourse						
<16 years	51/417 (12.2)	16/51 (31.4)	ref			
16-18 years	217/417 (52.0)	58/217 (26.7)	0.80 (0.41-1.55)	0.51		
≥18 years	147/417 (35.30)	44/147 (29.9)	0.93 (0.47-1.86)	0.85		
Lifetime sexual partners						
1	84/417 (20.1)	17/84 (20.2)	ref		ref	
2	126/417 (30.2)	30/126 (23.8)	1.23 (0.63-2.41)	0.54	0.90 (0.44-1.85)	0.93
≥3	206/417 (49.4)	72/206 (35.0)	2.12 (1.16-3.89)	**0.02**	1.20 (0.60-2.39)	0.67
Sexual partners in 12 months						
0	124/417 (29.7)	24/124 (19.4)	ref		ref	
1	254/417 (60.9)	82/254 (32.3)	1.99 (0.35-1.40)	**0.01**	1.16 (0.57-2.36)	0.67
≥2	38/417 (9.1)	13/38 (34.2)	2.17 (0.97-4.84)	**0.06**	0.89 (0.31-2.56)	0.83
Sexual partners in the last month						
0	200/417 (48.0)	44/200 (22.0)	ref		ref	
≥1	216/417 (51.8)	75/216 (34.7)	1.89 (1.21-2.92)	**0.004**	1.80 (0.68-4.79)	0.24
Used condoms during last sexual intercourse						
No	292/417 (70.0)	75/292 (25.7)	ref		ref	
Yes	121/417 (29.0)	43/121 (35.5)	1.60 (1.01-2.52)	**0.05**	1.07 (0.64-1.79)	0.8
Frequency of vaginal sex past 1 month						

0	220/417 (52.8)	52/220 (23.6)	ref		ref	
1-3 times	133/417 (31.9)	40/133 (30.1)	1.39 (0.86-2.25)	0.18	0.73 (0.28-1.91)	0.52
≥4 times	61/417 (14.6)	26/61 (42.6)	2.40 (1.32-4.35)	**0.004**	1.13 (0.40-3.22)	0.81
Frequency of anal sex past 1 month						
0	386/417 (92.6)	109/386 (28.2)	ref			
≥1 time	21/417 (5.0)	7/21 (33.3)	1.27 (0.50-3.23)	0.62		
Using any contraception with current partner						
No	258/417 (61.9)	62/258 (24.0)	ref			
Yes	154/417 (36.9)	57/154 (37.0)	1.86 (1.20-2.87)	**0.01**		
Method of contraception						
None/implant/ligation etc.	265/417 (5.4)	62/265 (23.4)	ref			
Injectables/Birth control pill	77/417 (18.5)	27/77 (35.1)	1.77 (1.02-3.06)	**0.04**		
Condoms	70/417 (16.9)	30/70 (42.9)	2.46 (1.41-4.27)	**<0.01**		
Age at first contraceptive use						
<16 years	5/417 (1.2)	1/5 (20.0)	ref			
16-18 years	21/417 (5.0)	6/21 (28.6)	1.60 (0.15-17.41)	0.7		
≥18 years	122/417 (29.3)	48/122 (39.3)	2.59 (0.28-23.91)	0.4		
Vaginal discharge (self-reported)						
No	192/417 (46.0)	49/192(25.5)	ref			
Yes	224/417 (53.7)	70/224(31.3)	1.33 (0.86-2.04)	0.2		
Frequency of vaginal discharge (self-reported)						
No vaginal discharge	192/417 (46.0)	49/192 (25.5)	ref		ref	
Current/last week	64/417 (15.4)	27/64 (42.2))	2.13 (1.18-3.85)	**0.01**	1.67 (0.88-3.15)	0.12
More than a week and less than 6 months	55/417 (13.2)	17/55 (30.9)	1.31 (0.68-2.52)	0.43	1.03 (0.50-2.13)	0.93
More than or equal to 6 months	103/417 (24.7)	24/103 (23.3)	0.89 (0.51-1.55)	0.67	0.73 (0.40-1.35)	0.32
Cytology						
Normal	373/417 (89.5)	97/373 (26.0)	ref		ref	
Abnormal	41/417 (9.8)	22/41 (53.7)	3.29 (1.71-6.35)	**<0.01**	2.83 (1.39-5.76)	**0.04**

HR-HPV: High-risk Human papillomavirus; **OR**: odds ratio; **CI**: confidence interval; **ref**: reference; **n**: number of women responded, **N**: total number of study participants.

2.4 DISCUSSION

This appears to be the first study reporting HR-HPV prevalence and risk factors among women attending a community-based clinic in rural Eastern Cape, South Africa. This is a cross-sectional study so causality and temporal relationships cannot be inferred. The study demonstrates a relatively high overall HR-HPV prevalence (28.5%) particularly, among HIV-positive women (40.6%). High HR-HPV prevalence and ineffective cervical screening programmes in a country with high burden of HIV, like South Africa, are major public health challenges. HIV-positive women are more likely to have HR-HPV persistent infection and progress to develop cervical cancer as compared to HIV-negative women (Dartell et al. 2012; McDonald et al. 2014; Moodley et al. 2006). The HR-HPV infection rate observed in the present study is higher than that reported in other African studies using HC-2 such as rural and urban Nigeria (15.6%) (Pimentel et al. 2013), rural Ethiopia (16.0%) (Leyh-Bannurah et al. 2014), rural and urban Tanzania (20.1%) (Dartell et al. 2012) and urban South Africa (20.7%) (McDonald et al. 2012). However, HR-HPV prevalence detected in this study is similar to that reported among women from rural Mali (23%) (Schluterman et al. 2013).

It is important to note that, in this study, being HR-HPV-positive was not influenced by level of education. This aspect has been previously reported to be significantly associated with HPV, as women with low level of education have been found to be more likely to engage in unprotected sexual activities (Mitchell et al. 2014). The present study show that multiple lifetime sexual partners (≥3), multiple sexual partners in the last month (≥1), sexual partners in the past 12-months and vaginal discharge at the time of the study or in the week before the study, were the most significant risk factors of HR-HPV infection. These findings confirm the current evidence on association of sexual behaviour and HPV infection (Winer et al. 2016; Nielsen et al. 2009). A study showed that women who reported having one or more high-risk sexual behaviours were almost three times more likely to have an incident detectable HR-HPV infection compared to those who reported not being recently sexually active (Winer et al. 2016). Sexual behaviour as the risk factor of HR-HPV infection played a significant role in this population. This highlights the need to emphasize the significance of implementing educational programs about HPV infection and risk reduction. There sexual partner and vaginal discharge variables and association with HR-HPV is only seen on univariate analysis.

HIV infection was found as an independent risk factor for HR-HPV in this study. Previous studies have reported HIV infection as the significant risk factor for HR-HPV infection (Dartell et al. 2012; Leyh-Bannurah et al. 2014; McDonald et al. 2012). This may be due to the effect of immunosuppression that causing reduced rates of HPV clearance, which may influence high rates of HPV infection, persistent HPV infection or reactivation of latent HPV (Strickler et al. 2005; Mbulawa et al. 2012). Mbulawa et al. (2012) showed that HIV-positive women were three times as likely to have a new HPV infection as compared to HIV-negative women (Mbulawa et al. 2012). Similarly, the decline of CD4 counts among HIV-positive women also influences the HPV prevalence (Mbulawa et al. 2010). A South African study found HR-HPV prevalence to be more than two-fold higher in older HIV-positive women (30 years or more) compared to HIV-negative (McDonald et al. 2014), relative to that found in our study (50 years or more). HR-HPV viral load was significantly higher among HIV-positive women with normal cytology and abnormal cytology. Since a high viral load has been associated with persistent HPV infection, studies suggest that HPV viral load could be used as the biomarker for predicting the progression of cervical lesions and an indicator for a follow-up after treatment of cervical lesions (Shen et al. 2014; Xi et al. 2011).

There are different ways in which those with HR-HPV-positive results can be managed, including referring these women to a colposcopy clinic or screen-and-treat where the health care worker would treat all the HR-HPV-positive women. In developed countries, HR-HPV-positive women are referred to colposcopy clinics for follow-up; however this would be difficult in South Africa due to lack of resources for cervical cancer screening and treatment (Denny and Kuhn 2017). South Africa has system barriers whereby there is a limited number of colposcopy system that are only available in urban areas, resulting in a long waiting list for treatment (Denny and Kuhn 2017; Katz et al. 2016). According to a South African study, screen (HPV testing)-and-treat is safe and effective and influences the prevalence of CIN. The study found that CIN2+ cases were 73% lower when using an HPV-based rather than a Visual Inspection Acetic acid (VIA) based screen-and-treat after 36 months follow-up (Denny et al. 2010). For HPV testing, there women were screened with HC-2 assay and cryotherapy was performed by freezing using nitrous oxide in women based on their screening test result. If the screen-and-treat approach was to be initiated in this rural community, the observed

28.5% HR-HPV-positive women would be treated. However, treating 28.5% of women would be challenging, and further resources and training would be needed before implementation. Another possibility is to introduce a triage system to identify those women at the highest risk of disease for treatment. In a randomised study done in Sweden, HR-HPV-positive women were rescreened with HPV testing after 4-6 months, and 71% of women were found to have persistent HR-HPV (Gustavsson et al. 2018). The cumulative prevalence of CIN2+ was much higher in women with persistent infection (20.2 per 1,000 women) compared to those with one HPV test (10.8 per 1000 women). HPV-positive women in South Africa may need to be triaged for treatment to target those at high risk of cervical disease. Further studies are needed in order to identify biomarkers for cervical lesions that do not need treatment (Gustavsson et al. 2018).

Such scenarios, when implemented into the healthcare system, would help improve the standard of care, overcome the barriers to cervical cytology screening, and improve health outcomes of women in the region. Eastern Cape Province has the poorest healthcare system, with limited resources and a lack of trained clinicians in public sectors to evaluate of high-risk cervical lesions and the treatment. Different screening methods that can be utilised in cervical cancer screening program. Previous studies have estimated the cost of initial cervical screening in South Africa as $36.50 for HPV DNA testing, $12.61 for liquid cytology, $9.75 for conventional cytology and $4.383 for VIA (Dreyer et al. 2019; Lince-Deroche et al. 2015). Clinically approved HPV DNA tests such as the HC-2 assay are relatively expensive compared to other HPV tests (such as careHPV assay) and VIA (Lince-Deroche et al. 2015; Katanga et al. 2019; Qiao et al. 2008). VIA is the most cost-effective test but has low performance. A low-cost HPV test is desirable for developing countries.

In this study, HPV infection was detected by HC-2 assay. This assay was selected because of its high clinical sensitivity and specificity. The clinical sensitivity is linked to higher copy numbers; therefore HC-2 was set up to detect a copy number that correlated with disease. The HC-2 assay is approved by the US FDA as a primary test for cervical cancer screening test and widely used as reference standard for HPV testing (Ejegod et al. 2016; Salazar et al. 2019). However, the use of HC-2 in this study could be the limitation, a PCR based assay could have

increased the HR-HPV prevalence. It is acknowledged that the study population were from one community health clinic and does not represent the population of rural Eastern Cape Province of South Africa; therefore, it cannot be used to generalise the whole population of rural Eastern Cape Province. Despite this limitation, the data reported in this report remain important and there is currently no other report on HR-HPV prevalence in this population.

2.5 CONCLUSION

HIV infection and sexual behaviour impact on the prevalence of HR-HPV. There sexual partner and vaginal discharge variables and association with HR-HPV is only seen on univariate analysis. The relatively high HR-HPV burden is an important factor when examining the feasibility of HPV based screen-and-treat approaches for this region. This report will inform health policymakers on HPV prevalence in this population and contribute to discussions on use of HPV testing as primary cervical cancer screening test in the South Africa.

CHAPTER 3: Distribution of human papillomavirus (HPV) genotypes in HIV-negative and HIV-positive women with cervical intraepithelial lesions in the Eastern Cape Province, South Africa

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ABSTRACT

Background: South African women have a high rate of cervical cancer cases, but there are limited data on human papillomavirus (HPV) genotypes in cervical intraepithelial neoplasia (CIN) in the Eastern Cape province, South Africa.

Method: A total of 193 cervical specimens with confirmed CIN from women aged 18 years or older, recruited from a referral hospital, were tested for HPV infection. The cervical specimens, smeared onto FTA cards, were screened for 36 HPV types using an HPV direct flow kit.

Results and conclusion: HPV prevalence was 93.5% (43/46) in CIN2 and 96.6% (142/147) in CIN3. HIV-positive women had a significantly higher HPV prevalence than HIV-negative women (98.0% vs. 89.1%, *p*=0.012). The prevalence of multiple types was significantly higher in HIV-positive than HIV-negative women (*p*=0.034). The frequently detected genotypes were HPV35 (23.9%), HPV58 (23.9%), HPV45 (19.6%), and HPV16 (17.3%) in CIN2 cases, while in CIN3, HPV35 (22.5%), HPV16 (21.8%), HPV33 (15.6%), and HPV58 (14.3%) were the most common identified HPV types, independent of HIV status. The prevalence of HPV types targeted by the nonavalent HPV vaccine was 60.9% and 68.7% among women with CIN2 and CIN3, respectively, indicating that vaccination would have an impact both in HIV-negative and HIV-positive South African women, although it will not provide full protection in preventing HPV infection and cervical cancer lesions.

3.1. INTRODUCTION

Human immunodeficiency virus (HIV) infection is highly predominant in Africa. Globally, approximately 25.7 million people live with HIV infection, of which 80% (20.7 million) reside in Eastern and Southern Africa, as reported in 2019 (Avert 2019; WHO 2019). Of the African countries, South Africa has the largest population affected by HIV infection, with 7.5 million people living with HIV and 200,000 new infections reported in 2019 (UNAIDS 2019). HIV prevalence is estimated to be 19.0% among women aged 15-49 years (UNAIDS 2019; WHO 2019). Among the nine provinces in South Africa, the prevalence of HIV infection for adult women aged 15-49 years ranges from 12.6% to 27.0%, with high rates observed in Kwazulu-Natal province (27.0%), Free State province (25.5%), and Eastern Cape Province (25.2%) (Human Sciences Research Council 2018).

An interaction between HIV infection and specific cancers has been established. Cervical cancer is one of the three cancers established as AIDS-defining cancers (Blattner and Nowak 2013). South Africa ranked as the country with the fourth highest number of cervical cancer cases among HIV-positive women (63.4%) in 2018 (Stelzle et al. 2020). The incidence rate of cervical cancer was estimated to be 506 per 10,000 person-years among HIV-positive South African women in 2017 (Rohner et al. 2017). Cervical cancer arises from CIN stages 1-3 and is causally associated with genital human papillomavirus (HPV) (zur Hausen 2002; Jemal et al. 2011). HIV-positive women have an increased burden of genital HPV acquisition, high-risk (HR) HPV persistent infection, multiple infections of HR-HPV, and precancerous lesions compared to HIV-negative women (Liu et al. 2018; Marembo, Dube Mandishora, and Borok 2019). Studies suggest that this results from immune suppression and low CD4 cell count (Liu et al. 2018; Kriek et al. 2016).

Persistent infection with HPV16, HPV18, and other HR-HPV genotypes is the most significant risk factor for developing cervical lesions and cervical cancer (Schettino et al. 2014). HPV16/18 are essential HR-HPV types that significantly contribute to cervical cancer disease progression (Khan et al. 2005). There are different strategies implemented to prevent preinvasive lesions and cervical cancer, mainly through HPV vaccination and cervical cancer screening. The type-specific HPV vaccines, namely, bivalent (HPV16/18), quadrivalent (HPV16/18/6/11), and

nonavalent (HPV16/18/6/11/31/33/45/52/58), have been introduced in more than forty countries, both developing and developed (Joura et al. 2015; Harper and Vierthaler 2011; Markowitz et al. 2012). These vaccines are offered to adolescent and young women aged 9–26 years (Harper and Vierthaler 2011; Joura et al. 2015). In South Africa, a national school-based vaccination campaign for the bivalent HPV vaccine was implemented to target public school girls aged nine years in grade 4. An uptake of the bivalent HPV vaccine ranging from 87% to 92% was positively attained among South African girls (Delany-Moretlwe et al. 2018; Botha et al. 2015). However, cervical cancer screening among older women is still necessary, as they are beyond the targeted age for receiving the HPV vaccine and are more likely to have been infected with HPV.

Molecular HPV DNA testing has been implemented as the alternative to non-molecular testing for cervical cancer, particularly cytology testing (Segondy et al. 2016). HPV testing is utilised in various strategies for screening, such as triage, co-testing, or HPV testing alone (Cuzick et al. 2015), and has high sensitivity but low specificity for the detection of CIN2/3 (Firnhaber et al. 2013; Ronco et al. 2014).

There is limited information on the epidemiology of HPV types in women with preinvasive cervical lesions from the Eastern Cape province. It is essential to investigate the prevalence of HPV genotypes and their distribution among women with different immune statuses and confirmed CIN histology results in this region. Therefore, these data will help achieve better understanding of the HR-HPV types involved in cervical lesions and cervical cancer cases. Furthermore, this information will contribute to discussions about implementing strategies for cervical screening and monitoring HPV types that are not present in the current vaccines to reduce cervical cancer disease in this population. Our study aims at investigating the distribution of HPV genotypes among HIV-positive and HIV-negative women with cervical intraepithelial lesions from Eastern Cape, South Africa.

3.2. MATERIAL AND METHODS

3.2.1 Study population

The study obtained ethical approval from the Human Ethics Committees of the University of Cape Town (UCT, HREC reference 615/2017), Walter Sisulu University (016/2017), and

Eastern Cape Department of Health (EC reference 2017RPO_484). The recruitment procedure for this study was reported previously (Taku et al. 2020a). Briefly, between September 2017 and March 2019, cervical specimens were collected among women referred to the Nelson Mandela Academic Hospital Gynaecology Outpatient Clinic located in the OR Tambo municipality area in the Eastern Cape Province, South Africa. A total of 193 women were recruited, aged ≥18 years, with atypical squamous cells of undetermined significance (ASCUS), atypical squamous cells cannot exclude high-grade lesions (ASC-H), atypical glandular cells, not otherwise specified (AGC-NOS), low-grade squamous intraepithelial lesions (LSIL), and high-grade squamous intraepithelial lesions (HSIL). The cervical specimens were collected by a study nurse using a Viba-brush (Rovers Medical Devices B.V., 5347 KV Oss, Netherlands), smeared onto FTA cards (GE Healthcare, Amersham place little Chalfont, Buckinghamshire HP7 9NA, UK), and shipped at room temperature to UCT. There cervical samples were first taken for the study and followed by the biopsy for further evaluation. The cervical biopsy was collected for histopathology and was performed by the National Health Laboratory Service. Based on the histopathology results, 46 women with CIN2 and 147 with CIN3 were included in this study. All eligible women provided signed consent forms for participation, collection of biological specimens and storage of their biological specimens for further laboratory-based studies.

3.2.2 Detection of HPV genotypes

DNA elution of cervical specimens from FTA cards was done following the procedure previously described (Taku et al. 2020a; Gustavsson et al. 2011). Four microlitres of extracted DNA was used for HPV testing. Detection of HPV genotypes was performed using an HPV direct flow chip kit on a Hybrispot machine (Master Diagnostica, Granada, Spain) following the manufacturer's procedure. The HPV direct flow chip protocol is a PCR-based method based on the amplification of a viral DNA fragment, followed by hybridisation onto a membrane chip using the amplified PCR products. The chip membrane contains DNA control, hybridisation control, PCR control, and probes for genotype specific HPV detection. The assay detects 36 HPV genotypes (low-risk HPV: 6, 11, 40, 42, 43, 44, 54, 55, 61, 62, 67, 69, 70, 71, 72, 81, 84, and 89 (C6108) and high-risk HPV:16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73 and 82). Each chip membrane's results were captured by a camera and analysed

automatically using HybriSoft software (Master Diagnostica, Granada, Spain) (Herraez-Hernandez et al. 2013).

3.2.3 Identification of HR-HPV using *hpVIR* real-time PCR

The eluted DNA from specimens on FTA cards was tested for HR-HPV using the clinically validated *hpVIR* real-time PCR assay (Gustavsson et al. 2019). *hpVIR* assay detects 12 HR-HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and the analysis was performed as previously described (Gustavsson, Juko-Pecirep, et al. 2009). Some HR-HPV types (16, -31, -35, -39, -51, -56, and -59) are identified as individual types, while HPV18 and -45, and HPV33, -52 and -58 are detected as two groups. The *hpVIR* assay also detect a human single copy house-keeping gene encoding *Homo sapiens* hydroxymethylbilane synthase (HMBS; GenBank accession no.M9523.1). This serves as a control for the integrity of the eluted DNA and interpretation of the results for HPV-negative samples. The *hpVIR* assay has a cut-off of for HMBS single copy gene, and 10 copies per PCR of HPV for a positive HR-HPV type (Gustavsson et al. 2019).

3.2.4 Statistical analysis

All data and statistical analyses were done using GraphPad Prism v6.01 (GraphPad Software, Inc., San Diego, CA). The chi-squared test was used to determine a statistical difference between HPV infection and variables. A variable was considered significant if the *p*-value was < 0.05.

3.3. RESULTS

3.3.1. Description of study participants

The median age of women was 40 (IQR: 33-48) years. A high number of women were HIV-positive (76.2%) and never smoked (93.3%), and half of the women had their first sexual experience at the age of 16-18 years (53.9%) (Table 3.1). Women were more likely to have ≥3-lifetime sexual partners (62.7%), with a high proportion having high-grade squamous lesions on cytology testing (75.1%) (Table 3.1).

Table 3.1: Demographic and behavioural characteristics of study participants.

Variables	% (n/N)
Age in years: Median (IQR)	40 (33-48)
HIV Status	
No	23.8% (46/193)
Yes	76.2% (147/193)
Age categories	
18-29 years	11.4% (22/193)
30-39 years	35.2% (68/193)
40-49 years	35.2% (68/193)
≥50 years	18.1% (35/193)
Highest level of education attained	
Never/ primary	29.5% (57/193)
High school/university	70.5% (136/193)
Household income	
< $139,36	75.1% (145/193)
≥ $139,36	22.8% (44/193)
Smoking status	
Never	93.3% (180/193)
Former/current smoker	6.2% (12/193)
Age at first sexual experience	
<16 years	21.2% (41/193)
16-18 years	53.9% (104/193)
≥18 years	24.9% (48/193)
Lifetime sexual partners	
1	15.0% (29/193)
2	21.8% (42/193)
≥3	62.7% (121/193)
Cytology	
ASCUS/ASCU-H/AGC-NOS	13.5% (26/193)
LSIL	9.3% (18/193)
HSIL	75.1% (145/193)

ASCUS: Atypical Squamous Cells of Undetermined Significance; **ASC-H**: Atypical Squamous Cells Cannot Exclude High-Grade Lesions; **AGC-NOS**: Atypical Glandular Cells, Not Otherwise Specified; **LSIL**: Low-Grade Squamous Intraepithelial Lesions; **HSIL**: High-Grade Squamous Intraepithelial Lesions. **n**: number of women responded, **N**: total number of study participants

3.3.2. HPV prevalence according to HIV status

Of the 193 women screened, 93.5% (43/46) with CIN2 and 96.6% with CIN3 had an HPV infection. HIV-positive women had a significantly higher prevalence of any HPV infection compared to HIV-negative women (98.0% vs. 89.1%, *p*=0.012) (Table 3.2). HIV-negative women were almost 2-times more likely to have only a single HPV infection compared to HIV-positive women, although there was no statistical significance (OR: 1.45; 95%CI: 0.735-2.867, *p*=0.282, Table 3.2). However, HIV-positive women had a significantly higher HPV prevalence compared to HIV-negative women (65.3% vs. 47.8%, *p*=0.034, Table 2). For multiple

infections, the median of HPV types was 2 (range: 2-11). When stratified by HIV status, there was no significant distinction between HIV-negative and HPV-positive women (Figure 3.1).

Table 3.2: Prevalence of HPV infection according to HIV status.

Variables	HIV-negative, N=46	HIV-positive, N=147	OR (95%CI)	*p*-value
Any types	89.1% (41/46)	98.0% (144/147)	0.17 (0.039-0.745)	0.012
Single infection	41.3% (19/46)	32.7% (48/147)	1.45 (0.735-2.867)	0.282
Multiple infection	47.8% (22/46)	65.3% (96/147)	0.49 (0.249-0.953)	0.034
HR-HPV types	82.6% (38/46)	87.1% (128/147)	0.71 (0.286-1.738)	0.446
Probable HR-HPV types	17.4% (8/46)	30.6% (45/147)	0.60 (0.260-1.394)	0.233
LR-HPV	39.1% (18/46)	44.2% (65/147)	0.81 (0.413-1.594)	0.543

HR-HPV: high-risk human papillomavirus; **LR-HPV**: low-risk human papillomavirus; **OR**: Odds Ratio; **CI**: Confidence Intervals.

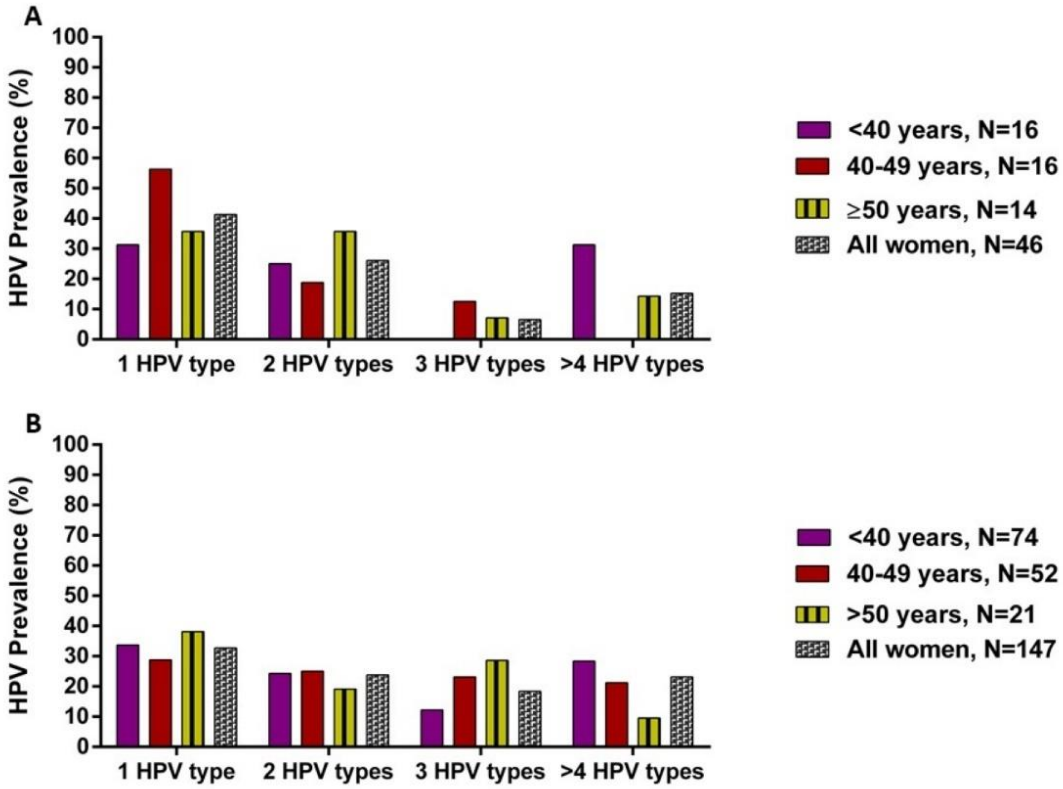

Figure 3.1: Distribution of single and multiple HPV types according to age in HIV-negative (a) and HIV-positive (b) women with cervical intraepithelial lesions.

3.3.3. HPV distribution according to cervical intraepithelial lesions and HIV status

Women with CIN2 were more likely to be infected with two HPV types (Figure 3.2A). The most frequently detected HPV types were HPV35 (23.9%), HPV58 (23.9%), HPV45 (19.6%), and

HPV16 (17.3%) (Table 3.3). Among single HPV infections, HPV35 and HPV16 were more frequent in HIV-negative women, while HPV16 and HPV52 were detected in HIV-positive women (Figure 3.3). For multiple HPV infections, HPV35, HPV58, and HPV45 were common in HIV-positive wom-en, whereas HPV35, HPV58, and HPV16 were frequently detected among HIV-negative women (Figure 3.3).

Women with CIN3 were more likely to be infected with two HPV types (Fig-ure 3.2B). Their commonly identified HPV types were HPV35 (22.5%), HPV16 (21.8%), HPV33 (15.6%), and HPV58 (14.3%) (Table 3.3). In single infections, HPV16, HPV35, and HPV33 were mostly observed in HIV-negative and HIV-positive women (Figure 3.4). However, the most detected HPV types in multiple infections were HPV16, HPV35, and HPV66 in HIV-negative, whereas HPV16, HPV35, and HPV45 were observed among HIV-positive women (Figure 3.4).

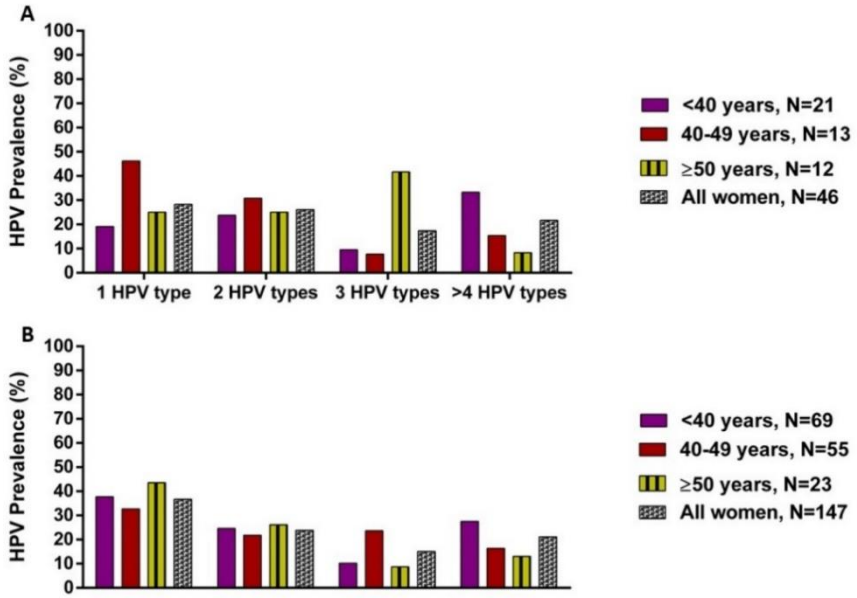

Figure 3.2: Distribution of single and multiple HPV types according to age in women with CIN2 (a) and CIN3 (b).

Table 3.3: Distribution of HPV genotypes among women with CIN2 and CIN3 from a referral hospital.

HPV types	All women (CIN2/3) % (n/N)	CIN2 % (n/N)	CIN3 % (n/N)
16	**20.7% (40/193)**	**17.4% (8/46)**	**21.8% (32/147)**
18	8.8% (17/193)	10.9% (5/46)	8.8% (12/147)
31	11.4% (22/193)	8.7% (4/46)	12.2% (18/147)
33	13.0% (25/193)	4.4% (2/46)	**15.6% (23/147)**
35	**22.8% (44/193)**	**23.9% (11/46)**	**22.5% (33/147)**
39	6.2% (12/193)	2.2% (1/46)	7.5% (11/147)
45	**15.0% (29/193)**	**19.6% (9/46)**	**13.6% (20/147)**
51	6.7% (13/193)	6.5% (3/46)	6.8% (10/147)
52	13.5% (26/193)	**15.2% (7/46)**	12.9% (19/147)
56	8.3% (16/193)	4.4% (2/46)	9.5% (14/147)
58	**16.6% (32/193)**	**23.9% (11/46)**	**14.3% (21/147)**
59	2.6% (5/193)	4.4% (2/46)	2.0% (3/147)
26	4.1% (8/193)	4.4% (2/46)	4.1% (6/147)
53	0.5% (1/193)	0.0% (0/46)	0.7% (1/147)
66	8.3% (16/193)	8.7% (4/46)	8.2% (12/147)
68	4.1% (8/193)	4.4% (2/46	4.1% (6/147)
73	2.6% (5/193)	4.4% (2/46)	2.0% (3/147)
82	10.4% (20/193)	6.5% (3/46)	11.6% (17/147)
6	6.2% (12/193)	6.5% (3/46)	6.1% (9/147)
11	4.7% (9/193)	0.0% (0/46)	6.1% (9/147)
40	3.6% (7/193)	2.2% (1/46)	4.1% (6/147)
42	7.3% (14/193)	8.7% (4/46)	6.8% (10/147)
43	2.1% (4/193)	0.0% (0/46)	2.7% (4/147)
44/55	10.9% (21/193)	17.4% (8/46)	8.8% (13/147)
54	3.6% (7/193)	6.5% (3/46)	2.7% (4/147)
61	0.5% (1/193)	0.0% (0/46)	1.4% (1/147)
62/81	15.0% (29/193)	19.6% (9/46)	13.6% (20/147)
70	4.1% (8/193)	6.5% (3/46)	3.4% (5/147)
71	4.7% (9/193)	2.2% (1/46)	5.4% (8/147)
72	3.6% (7/193)	6.5% (3/46)	2.7% (4/147)

HPV: human papillomavirus; **CIN**: cervical intraepithelial neoplasia**; n**: number of women responded, **N**: total number of study participants. Bold indicates the most dominant HPV types.

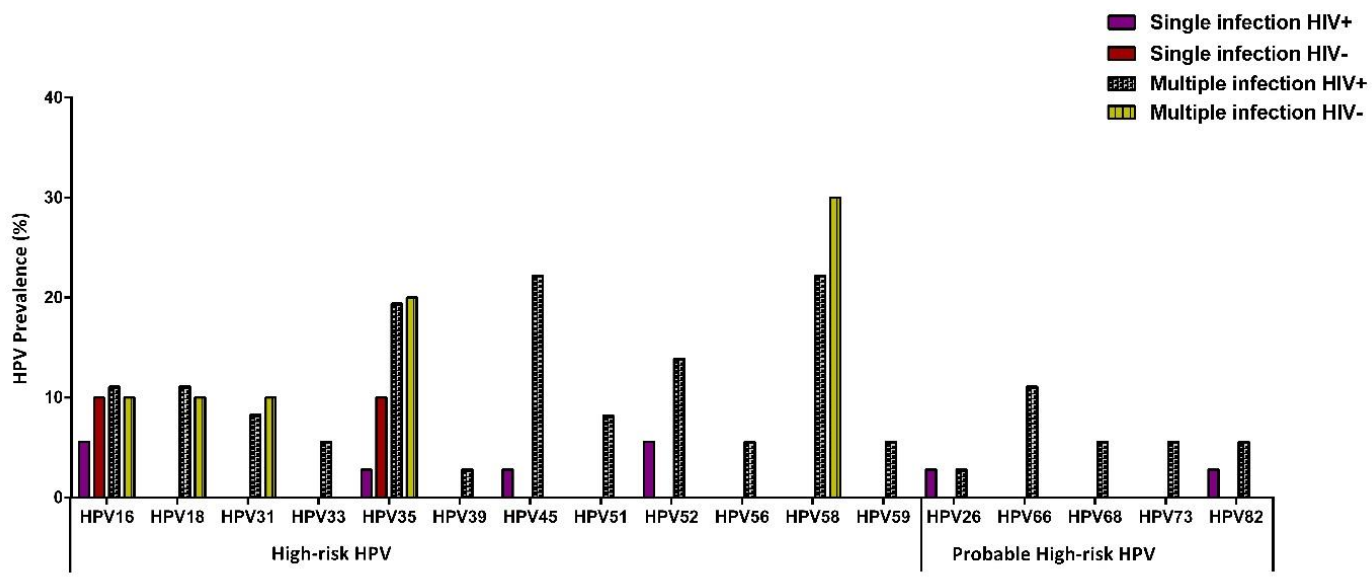

Figure 3.3: Distribution of HPV types among women with CIN2 in single and multiple according to HIV status.

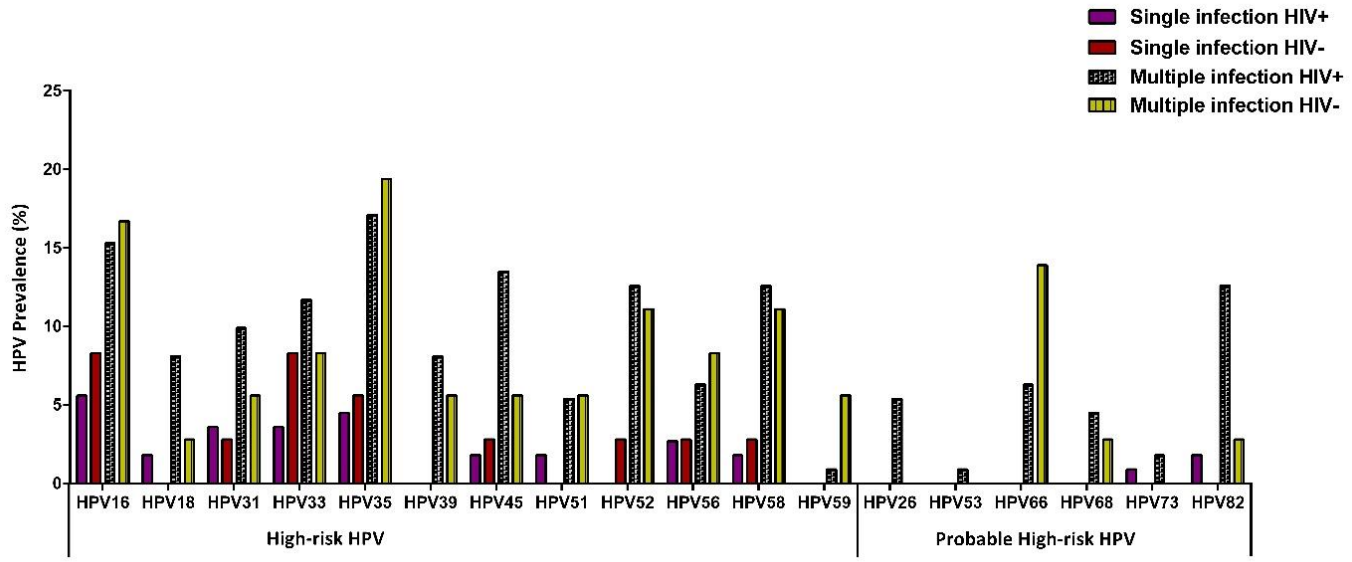

Figure 3.4: Distribution of HPV types among women with CIN3 in single and multiple according to HIV status.

3.3.4. HPV prevalence according to vaccine HPV types

The prevalence of bivalent vaccine HPV types (HPV16/18) increased from 20.0% of CIN2/HIV-negative to 25.0% of CIN2/HIV-positive and from 27.8% of CIN3/HIV-negative to 29.7% of CIN3/HIV-positive, with no statistical significance (p=1.000 and p=0.823, respectively) (Figure 3.5). For quadrivalent HPV types (HPV6/11/16/18), the positivity increased from 20.0% for

CIN2/HIV-negative to 30.5% for CIN2/HIV-positive and from 33.3% for CIN3/HIV-negative to 36.0% for CIN3/HIV-positive (p=0.700 and p=0.768, respectively) (Figure 3.5). CIN2/HIV-positive women had a higher prevalence of nonavalent HPV types than CIN2/HPV-negative, with no statistical significance (66.7% vs. 40.0%, p = 0.157). However, CIN3/HIV-negative women had a similar prevalence of nonavalent HPV types (HPV6/11/16/18/31/33/45/52/58) to CIN3/HIV-positive women (67.7% vs. 69.0%, p = 0.761) (Figure 3.5).

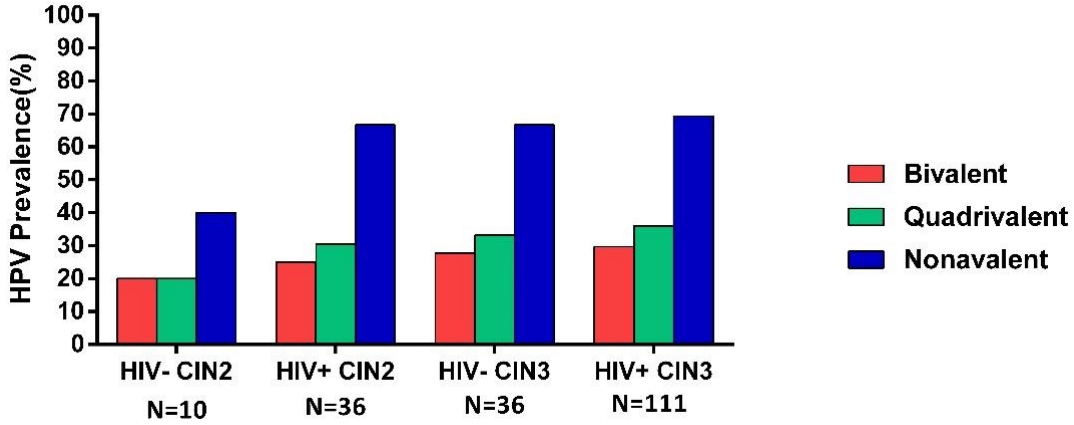

Figure 3.5: Prevalence of HPV vaccine types according to CIN2/3 and HIV status.

3.4 DISCUSSION

This study investigated the prevalence and distribution of HPV types among HIV-positive and HIV-negative women with high-grade precancerous cervical lesions. A high number of women in this study were HIV-positive (76.2%). A significantly high overall prevalence of any HPV infection (98% vs. 89%) and multiple infections (65% vs. 49%) was observed among HIV-positive compared to HIV-negative women with cervical intraepithelial lesions. This higher prevalence of multiple types of HPV infection among women with HIV infection agrees with other cohorts of women with high-grade lesions from Botswana and South Africa (Ramogola-Masire et al. 2011; McDonald et al. 2014; Van Aardt et al. 2016). However, in a South African study by Van Aardt and colleagues, the rate of multiple HPV infections among HIV-positive and HIV-negative women (81.3% vs. 64.4%) with confirmed CIN2/3 was higher compared to our study (Van Aardt et al. 2016). This difference could be attributed to the various assays used for HPV testing and the different study populations.

A high prevalence of HPV in women with CIN2 (93.5%) and CIN3 (96.6%) was observed in this study, which is expected as these women had abnormal cytology from the referral clinic. Similarly, a high HPV prevalence was reported in a global meta-analysis study, whereby HPV prevalence ranged from 86% to 93% in women with high-grade lesions (CIN2/3) (Guan et al. 2012). In the present cohort, HPV35 was the most predominant HPV type among participants with CIN2 lesions, while in those with CIN3 lesions, HPV16 and HPV35 were the most frequently detected HPV genotypes, either as a single HPV infection or multiple infections, regardless of HIV status. This observation was similar to other studies from South Africa and Kenya, where HPV16 and 35 were the most common genotypes in HIV-positive or HIV-negative women with high-grade squamous intraepithelial lesions or CIN2/3 (Menon et al. 2016; McDonald et al. 2014). However, a study among sex workers from Kenya showed that HPV52 was the most prevalent HPV type and more likely to be present as a single infection in women with severe lesions (HSIL/SCC) (Sweet et al. 2020). Furthermore, a recent cross-sectional study among women from four developed countries (Iceland, Norway, Sweden, and Denmark) reported a different distribution of HPV types, with HPV16, 31, and 52 present in CIN2 cases and HPV 16, 31, and 33 detected in CIN3 cases (Dovey de la Cour et al. 2019). The high occurrence of HPV35 in this population and other African studies of women with invasive cervical cancer suggests an interaction between HPV35 and cervical carcinogenesis (Pinheiro et al. 2020; Howitt et al. 2017; Castellsagué et al. 2008). Therefore, preventative strategies are needed, as the HPV35 genotype is present in up to 10% of sub-Saharan African women with invasive cervical cancer (Okolo et al. 2010; Denny et al. 2014; Clifford et al. 2016; Castellsagué et al. 2008) and not present in the current HPV vaccines.

Previously, cervical histological lesions were associated with one HPV type (van der Marel et al. 2015). However, in the present cohort, most high-risk HPV types and probable high-risk HPV types occurred as multiple HPV infections both in CIN2 and CIN3 cases. Multiple infections are reported as the risk factor of persistent infection and associated with high-grade CIN2/3 cases compared to a single infection (Schmitt et al. 2013). Women with multiple HPV types have been found to have larger cervical lesions and are associated with poor responses to cervical cancer treatment (Kaliff et al. 2018; Munagala et al. 2009). Kaliff et al. (2018) reported a significantly high recurrence rate of cervical cancer among women with

multiple HPV infections compared to a single HPV infection (44.0% vs. 24.0%) and a low cancer survival rate (Kaliff et al. 2018). Furthermore, the high prevalence of multiple HPV infections in the present study could be because HPV testing was performed on cervical cells instead of biopsy specimens. Therefore, it is not possible to determine which HPV types caused the lesion and which infected other parts of the cervix. HPV testing on biopsies eliminates the detection of multiple HPV infections, and multiple HPV infections are observed to be significantly lower in biopsies compared to exfoliated cells from invasive cervical cancers (De Vuyst et al. 2013; Clifford et al. 2016).

Interestingly, in our study, HPV16 was not the predominant HPV type, as it ranked fourth in CIN2 and second in CIN3 cases in the present cohort. A low prevalence of HPV16 has been observed in other sub-Saharan studies, while a high prevalence was observed in European studies (Bruni et al. 2010; Monsonego et al. 2015; Tornesello et al. 2014). These findings could be explained by the population being sampled from different geographical areas, host genetic difference/host immunogenic factors, and the biological interplay between HPV types (Hildesheim and Wang 2002). It is important to do a study based on cervical cancer biopsies to determine which HPV types are causally involved in cervical cancer in this community. The distribution of HPV types in this study are concerning, as there are many types that are not in the available vaccines.

Persistent HR-HPV infection is regarded as a significant factor in the development of cervical cancer lesions. However, in the current study, 4.2% (8/193) of women with CIN2/3 were negative for any HPV type, and seven of these samples were also negative when typed with *hpVIR* real-time PCR (Taku et al. 2020a). One specimen was positive on hpVIR real-time PCR for HR-HPV infection (HPV59) but had a low HPV copy number (11.3 copies) and viral titre (0.052). The observed negative results of HPV infection in women with high-grade lesions may suggest that it could result from sample storage, inadequate sampling, or low viral load. Alternatively, there may be novel HPV types causing the cancers that are not detected by the test used.

The currently available HPV vaccines are estimated to prevent 70-90% of cervical cancer cases (Serrano et al. 2014). The nonavalent HPV vaccine has been highly effective in preventing HPV infection and cancer diseases, with efficacies ranging between 90% and 100% (Garland et al. 2018; Joura et al. 2015; Huh et al. 2017; Ruiz-Sternberg et al. 2018). A study reported by Garland and colleagues (2018) in Asian women showed that nonavalent significantly decreased the risk of persistent infection, abnormal cytology, and diseases caused by specific HPV types targeted by this vaccine (Garland et al. 2018). In the present study, HPV vaccines could protect 20-69.0% of CIN2/3 cases in women with or without HIV infection. Therefore, the high prevalence of HPV types targeted by the nonavalent HPV vaccine suggests that introducing this HPV vaccine would be beneficial, as most precancerous lesions could have been prevented. However, the predominant HPV genotype (HPV35) in this population, which accounts for 24% in CIN2 and 23.0% in CIN3, is not covered by the nonavalent HPV vaccine.

Vaccines have been found to provide cross-protection against specific vaccine HPV types as well as some types that are not present in the vaccine. Numerous trial studies have reported that the bivalent vaccine offers a wider extent of cross-protection against nonvalent specific types (31/33/45/52/58), with less extensive cross-protection by Gardasil-9 (Kreimer et al. 2015; Skinner et al. 2016; Malagón et al. 2012). Studies have reported that bivalent showed substantial cross-protection against HPV31/33/45 but less so against HPV35 and HPV58 after seven to eight years post vaccination (Tsang et al. 2020; Kreimer et al. 2015; Bogaards et al. 2019). A study by Brown and colleagues among younger women aged 16-25 years vaccinated with the quadrivalent HPV vaccine showed a reduction of high-grade lesions (32.5%) related to ten non-vaccine HPV types (31/33/35/39/45/52/52/56/58/59) known to cause cervical cancer after 3.6 years of follow-up (Brown et al. 2009). Therefore, this suggests that cross-protection might have played a role and that the benefits of vaccination could include protection from clinically relevant HPV types not included in the vaccines (Brown et al. 2009). The cross-protection is related to the phylogenetic distance between the HPV types, as they are all closely related to vaccine types and found in the alpha-9 group (Bogaards et al. 2019). However, since cross-protection against HPV35 is observed to be less efficient compared to other HPV types, the addition of HPV35 to the next-generation HPV vaccine would improve the effectiveness of the HPV vaccine, especially in Africa.

5. Conclusions

We observed a significantly higher prevalence of HPV and multiple HPV infections in HIV-positive compared to HIV-negative women with cervical intraepithelial lesions. The distribution of HPV genotypes was similar between CIN2 and CIN3 cases independently of HIV status. The HPV nonavalent vaccine would have an impact on South African women, although it will not provide full protection in preventing HPV infection and cervical cancer lesions. Therefore, the high prevalence of the non-vaccine type (HPV35) underscores the need to incorporate this HPV type into the next HPV vaccines. This study also highlights the importance of introducing cervical cancer screening strategies to monitor non-vaccine HPV types.

CHAPTER 4: Acceptability of self- collection for human papillomavirus detection in the Eastern Cape, South Africa

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ABSTRACT

Background: Human papillomavirus (HPV) testing on vaginal self-collected and cervical clinician-collected specimens shows comparable performance. Self-sampling on FTA cards is suitable for women residing in rural settings or not attending regular screening and increases participation rate in the cervical cancer screening programme. We aimed to investigate and compare high-risk (HR)-HPV prevalence in clinician-collected and self-collected genital specimens as well as two different HPV tests on the clinician collected samples.

Method: A total of 737 women were recruited from two sites, a community health clinic (n=413) and a referral clinic (n=324) in the Eastern Cape Province. Cervical clinician-collected (FTA cards and Digene transport medium) and vaginal self-collected specimens were tested for HR-HPV using the *hpVIR* assay (FTA cards) and Hybrid Capture-2 (Digene transport medium).

Results: There was no significant difference in HR-HPV positivity between clinician-collected and self-collected specimens among women from the community-based clinic (26.4% vs 27.9%, *p*=0.601) or the referral clinic (83.6% vs 79.9%, *p*=0.222). HPV16, HPV35, and HPV33/52/58 group were the most frequently detected genotypes at both study sites. Self-sampling for HPV testing received a high positive response of acceptance (77.2% in the community-based clinic and 83.0% in referral clinic). The overall agreement between *hpVIR* assay and HC-2 was 87.7% (k=0.754).

Conclusion: The study found good agreement between clinician-collected and self-collected genital specimens. Self-collection can have a positive impact on a cervical screening program in South Africa by increasing coverage of women in rural areas, in particular those unable to visit the clinics and women attending clinics where cytology-based programs are not functioning effectively.

4.1 INTRODUCTION

Cervical cancer , is the third-ranking cause of cancer disease with an estimated 569,847 cases leading to 311,365 deaths in women globally in 2018 (Bruni et al. 2019b). The age-standardised incidence rate of cervical cancer varies worldwide from ≤2 to 75 per 100,000 women in different populations (Arbyn et al. 2020). In South Africa, cervical cancer is the leading cause of cancer in women of reproductive age (15-44 years) and the most important cancer in black women. About 12,983 new cervical cancer cases are diagnosed annually in South Africa (estimates for 2018) with an incidence of 31.7 per 100, 000 women (Bruni et al. 2019a; National Institute For Communicable Diseases Of South Africa 2020).

Cervical cancer is causally associated with infection by high-risk types of human papillomavirus (HPV) (Bruni et al. 2019b). Cervarix, which targets HPV16 and 18 (Arbyn, Xu, et al. 2018), is the current HPV vaccine used in school-based HPV vaccination programme in South Africa. Although this vaccine is expected to prevent at least 70% of cervical cancer, both vaccinated women and unvaccinated women still need to continue to be screened for cervical cancer (Arbyn, Xu, et al. 2018). Cervical cancer screening methods include Visual Inspection with Acetic acid (VIA), cytology-based (Pap smear or liquid-based cytology) and HPV DNA testing (Denny et al. 2015). Worldwide, cytology-based screening is still the most used screening test, although it has not been successfully implemented in African countries, including South Africa (Hoque, Hoque, and Kader 2008; WHO 2014b). In the Eastern Cape province of South Africa, cervical cancer screening coverage is <50%, which is below the national target of 70% coverage (Makura et al. 2016). Pap smear screening is not readily available to most of the women in rural South Africa, including Eastern Cape Province (Makura et al. 2016; Sibiya and Grainger 2007). In addition, while cervical cancer screening programmes may be available in some local clinics, the majority of women are not aware of the available services and not likely to participate (Nene et al. 2007; Waller et al. 2009; Daley et al. 2011).

HPV DNA testing is an effective cervical cancer prevention screening method and offers many advantages compared to cytology-based screening. This includes high sensitivity in predicting future precancerous lesions and cervical cancer, ability to perform self-collection of

specimens for HPV testing, and the possibility to increase of screening intervals for women with an HPV-negative test (>5-years) (Ogilvie et al. 2018; Kitchener 2019). A randomised study in India reported that a single HPV DNA test is more likely to lower the mortality rate of cervical cancer disease as compared to cytology-based screening or VIA (Sankaranarayanan et al. 2009). Australia and Netherlands have introduced vaginal self-sampling for HPV DNA testing in their routine screening program as an alternative screening method for non-attendees (Polman, de Haan, et al. 2019; Committee 2014) and shown increased participation (Arbyn, Smith, et al. 2018; Gök et al. 2010; Tranberg et al. 2018). Therefore, introducing a self-collection method might overcome barriers that limit the use of the Pap smear test and make cervical cancer screening accessible to more women.

Liquid-based transport medium is commonly used to transport and store cervical or vaginal specimens for HPV testing. FTA cards for storage of cervical or vaginal specimens, have several advantages over a liquid-based media. These include inclusion of a colour indicator to confirm that the sample is applied correctly, the samples become non-infectious once dried, long term stable storage at room temperature and easy transport (Gustavsson, Lindell, et al. 2009; Guan et al. 2013; Gustavsson et al. 2011; de Bie et al. 2011). Unlike the liquid-based transport medium, FTA cards are suitable for self-collection at home and can be transported via regular mail or other means not involving a cold-chain (Gustavsson et al. 2011). The process of extracting DNA from the FTA card is easy, quick and can be automated (Gustavsson et al. 2011; Gustavsson, Lindell, et al. 2009). Using the FTA card in combination with a clinically validated HPV DNA test has also been shown to work effectively for detection of HR-HPV infection (Geraets et al. 2013); with clinical specimens depicting up to 100% agreement of HR-HPV detection between FTA cards and liquid-based medium (Lenselink et al. 2009).

Due to the lack of infrastructure to perform Pap smear tests, cytology screening is not fully functional in rural settings. It is essential to implement a cervical cancer screening test that is easily accessible (such as self-collection) and simple to handle for women that do not have access to healthcare facilities offering regular cytology screening. In this study we aimed to a) determine the prevalence of HR-HPV genotypes in women attending a community-based clinic and a referral clinic in the Eastern Cape, b) investigate and compare the HR-HPV

prevalence in clinician-collected and self-collected genital specimens on FTA cards and to c) investigate attitudes to self-collection in women from the Eastern Cape.

4.2 MATERIALS AND METHODS

4.2.1 Study population and sample collection

This cross-sectional study was carried out from September 2017 to March 2019 at a community health clinic and the referral clinic for women with abnormal Pap smears within the OR Tambo district in the Eastern Cape Province of South Africa. A total of 413 women aged ≥30 years attending the community health clinic for cervical cancer screening or other reasons were recruited. Also, 324 women aged ≥18 years with abnormal cervical cytology and cervical cancer were recruited from the referral clinic. Written signed consent forms were obtained from all study participants, and sociodemographic data with other factors were obtained through a questionnaire. The ethical approval for this study was granted by the Human Research Ethics Committees of the University of Cape Town (UCT) (HREC reference 615/2017), Walter Sisulu University (reference 090/2016), and Eastern Cape Department of Health Ethics (EC reference 2017RP0_484).

Study participants provided three genital specimens. One clinician-collected cervical and one self-collected vaginal specimen were collected using Viba-brushes (Rovers Viba-Brush, Rovers Medical Devices) and applied onto indicating FTA elute cards (GE Health Care Life Sciences, Buckinghamshire, United Kingdom). A second clinician-collected cervical specimen was obtained using cervical brushes and stored in Digene transport medium (Qiagen, Gaithersburg, MD, USA). All specimens were kept at room temperature until shipped to the UCT HPV laboratory for analysis. Among participants recruited from the referral clinic, a cervical biopsy was collected and sent to the National Health Laboratory Service for histological analysis. The histology results were interpreted according to the guidelines of the International Agency for Research on Cancer (Sellors and Sankaranarayanan 2003).

4.2.2 Elution of DNA from FTA cards

DNA elution was performed as previously described (Gustavsson et al. 2011). Briefly, four 3.2 mm punches were obtained from each FTA card using the DBS Puncher Instrument (Perkin

Elmer Life and Analytical Sciences, Wallac, Oy, Finland) and collected in a 96-well plate. The punches were washed with sterile water and the DNA eluted in 70 µl water by incubating in a PCR machine at 95°C for 30 minutes. The DNA was stored at -20°C until further use.

4.2.3 Identification of HR-HPV using *hpVIR* real-time PCR

The eluted DNA from specimens on FTA cards was tested for HR-HPV using the clinically validated *hpVIR* real-time PCR assay (Gustavsson et al. 2019). *hpVIR* assay detects 12 HR-HPV genotypes (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) and the analysis was performed as previously described (Gustavsson, Juko-Pecirep, et al. 2009). Some HR-HPV types (16, -31, -35, -39, -51, -56, and -59) are identified as individual types, while HPV18 and -45, and HPV33, -52 and -58 are detected as two groups. The *hpVIR* assay also detect a human single copy house-keeping gene encoding *Homo sapiens* hydroxymethylbilane synthase (HMBS; GenBank accession no.M9523.1). This serves as a control for the integrity of the eluted DNA and interpretation of the results for HPV-negative samples. The *hpVIR* assay has a cut-off of for HMBS single copy gene, and 10 copies per PCR of HPV for a positive HR-HPV type (Gustavsson et al. 2019).

4.2.4 Detection of HR-HPV using Hybrid Capture-2

Cervical specimens in Digene transport medium were tested for 13 HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) using the Hybrid Capture-2 (HC-2) assay (Qiagen Inc., Gaithersburg, MD, USA) according to the manufacturer's protocol. A ratio of relative light units/cut-off ≥1 was considered positive while a ratio <1 was considered negative for HR-HPV types.

4.2.5 Statistical analysis

Data analysis was performed using GraphPad Prism v6.01 (GraphPad Software, Inc., San Diego, CA). Descriptive statistics (medians and interquartile range) and frequency distribution were used to describe the sociodemographic and other variables of study participants in the population. For statistical analysis, chi-squared test was used to determine the difference in estimated HR-HPV prevalence between self-collected and clinician-collected samples. A p-value <0.05 was considered significant. The overall agreement and HR-HPV genotype distribution agreement (percent agreement, kappa values with 95% Confidence Intervals)

were determined using the kappa statistic. The kappa values were interpreted using a standard method (http://www.graphpad.com/quickcalcs/Kappa2.cfm).

4.3 RESULTS

4.3.1 Description of study participants

Table 4.1 summarises the baseline demographics of study participants and other factors. Women from the community-based clinic had a median age of 46 years (IQR: 38-55), most were ≥50 years (42.4%), 37.3% were HIV-positive and the majority had been pregnant (96.6%). Almost half the women had ≥3-lifetime sexual partners (49.4%) and most of the women never smoked (94.4%). A high proportion of women had attended high school or university (73.6%), and the monthly household income was <$139,36 (71.2%).

Median age of the referral clinic women was 40.5 years (IQR: 33-49) and the majority were HIV-positive (71.3%). Half of the study participants had attended high school or university (54.3%) and 77.4% of women had a monthly household income of <$139,36. The majority of women had their first sexual experience at the age of 16-18 years (54.3%), with 60.8% of them having ≥3-lifetime sexual partners.

Table 4. 1: Demographic and behaviour characteristics of study participants.

	Community clinic	Referral clinic
Variables	**% (n/N)**	**% (n/N)**
HIV Status		
No	62.7 (259/413)	27.8 (90/324)
Yes	37.3 (154/413)	71.3 (231/324)
Missing	0.0 (0/413)	0.9 (3/324)
If yes, ARV's?		
No	3.9 (6/154)	1.3 (3/231)
Yes	96.1 (148/154)	98.3 (227/231)
Missing	0.0 (0/154)	0.4 (1/231)
Age categories		
18-29 years	……	13.0 (42/324)
30-39 years	31.5 (130/413)	33.3 (108/324)
40-49 years	26.2 (108/413)	31.2 (101/324)
≥50 years	42.4 (175/413)	22.5 (73/324)
Missing	0.0 (0/413)	0.0 (0/324)
Highest level of education attained		
Never/ primary	26.4 (109/413)	45.7 (148/324)
High school/university	73.6 (304/413)	54.3 (176/324)
Missing	0.0 (0/413)	0.0 (0/324)
Household income		
< $139,36	71.2 (294/413)	77.5 (251/324)
≥ $139,36	27.6 (114/413)	21.3 (69/324)
Missing	1.2 (5/413)	1.2 (4/324)
Smoking status		
Never	94.4 (390/413)	92.0 (298/324)
Former/current smoker	5.6 (23/413)	7.7 (25/324)
Missing	0.0 (0/413)	0.3 (1/324)
Ever drank alcohol		
No	91.0 (376/413)	81.5 (264/324)
Yes	9.0 (37/413)	18.2 (59/324)
Missing	0.0 (0/413)	0.3 (1/324)
Age at first sexual experience		
<16 years	12.1 (50/413)	19.4 (63/324)
16-18 years	52.5 (217/413)	54.3 (176/324)
≥18 years	34.9 (144/413)	26.2 (85/324)
Missing	0.5 (2/413)	0.0 (0/324)
Lifetime sexual partners		
1	20.3 (84/413)	15.1 (49/324)
2	30.0 (124/413)	23.8 (77/324)
≥3	49.4 (204/413)	60.8 (197/324)
Missing	0.2 (1/413)	0.3 (1/324)
Used condoms during last sexual intercourse		
No	70.0 (289/413)	65.4 (212/324)
Yes	29.1 (120/413)	33.6 (109/324)
Missing	0.9 (4/413)	1.0 (3/324)
Method of contraceptive, last time had sex		
None/implant/ligation etc	51.1 (211/413)	52.2 (169/324)
Injectables/birth control pill	21.1 (87/413)	20.7 (67/324)
Condoms	26.1 (108/413)	26.9 (87/324)
Missing	1.7 (7/413)	0.3 (1/324)
Using any contraception currently		
No	61.7 (255/413)	60.8 (197/324)
Yes	37.1 (153/413)	38.6 (125/324)
Missing	1.2 (5/413)	0.6 (2/324)
Pregnancy		
No	3.2 (13/413)	4.6 (15/324)
Yes	96.6 (399/413)	95.1 (308/324)
Missing	0.2 (1/413)	0.3 (1/324)

HIV: human immunodeficiency virus **ARV's**: Antiretrovirals, **n**: number of women responded, **N**: total number of study participants; **Missing**: not answered

4.3.2 HR-HPV prevalence and agreement between self-collected and clinician-collected specimen using *the hpVIR assay*

All specimens on FTA cards had sufficient amounts of genomic DNA (10 copies or more of the HMBS single copy gene) for the HPV test to be informative. For women recruited at the community-based clinic, there was no significant difference in HR-HPV prevalence between self-collected and clinician-collected samples [27.9 %(115/413) vs 26.4% (109/413), *p*=0.639]. HR-HPV positivity between self-collected and clinician-collected samples showed an agreement of 86.9%, with a kappa value of 0.669 [95% CI: 0.588-0.750, (Table 4.2)].

Among the referral clinic women, HR-HPV prevalence was slightly higher in clinician-collected versus self-collected specimens, but the difference was not significant [83.6% (271/324) vs 79.9% (259/324), *p*=0.222]. The observed overall agreement of HR-HPV infection between self-collected and clinician-collected samples was 91.4% [k=0.711, 95%CI: 0.610 to 0.811, (Table 4.2)].

Table 4. 2: Concordance between high-risk HPV in self-collected and clinician-collected genital specimens from the community and the referral clinics.

Community clinic	**Self-collected**				
		HR-HPV positive (%)	**HR-HPV negative (%)**	**Total (%)**	**% Agreement**
Clinician-collected	**HR-HPV positive (%)**	85 (20.6)	24 (5.8)	109 (26.4)	86.9 (k=0.669)
	HR-HPV negative (%)	30 (7.3)	274 (66.3)	304 (73.6)	
	Total (%)	115 (27.9)	298 (72.1)	413 (100.0)	

Referral clinic	**Self-collected**				
		HR-HPV positive (%)	**HR-HPV negative (%)**	**Total (%)**	**% Agreement**
Clinician-collected	**HR-HPV positive (%)**	251 (77.4)	20 (6.2)	271 (83.6)	91.4 (k=0.711)
	HR-HPV negative (%)	8 (2.5)	45 (13.9)	53 (16.4)	
	Total (%)	259 (79.9)	65 (20.1)	324 (100.0)	

HR-HPV: high-risk human papillomavirus, **k**-kappa value

4.3.3 HR-HPV genotypes and agreement between clinician-collection and self-collection using the *hpVIR assay*

Of the 413 women attending the community-based clinic, 19.1% of the self-collected and 20.4% samples of clinician-collected samples were infected with single HR-HPV types, and this difference was not statistically significant (*p*=0.601, Table 4.3). The prevalence of multiple HR-

HPV infections was not significantly different between self-collected and clinician-collected specimens (8.7% vs 5.8%, *p*=0.108). The most frequently detected HR-HPV genotypes were HPV16, HPV35, and the HPV33/52/58 group, both in self-collected and clinician-collected specimens (Figure 4.1). The agreement in the HR-HPV genotypes identified in self-collected and clinician-collected specimens ranged from moderate to almost perfect (k=0.571-0.888), with HPV39 (99.8%, k=0.888) and HPV31 (99.3%, k=0.838) showing almost perfect agreement (Table 4.4).

In the referral clinic, the distribution was similar when comparing HR-HPV genotypes in self-collected and clinician-collected samples. The HPV33/52/58 group, HPV16 and HPV35 were the most frequently detected HR-HPV genotypes in both collection groups (Figure 4.1). The prevalence of single HR-HPV infection was somewhat higher in clinician-collected specimens as compared to self-collected specimens, but the difference was not statistically significant [46.0% vs 42.3%, *p*=0.342, (Table 4.3)]. The HR-HPV prevalence of multiple infection was the same in self-collected and clinician-collected specimens [37.7% vs 37.7%, *p*=1.000, (Table 4.3)]. The concordance of the HR-HPV genotype distribution between collection groups showed kappa values ranging between 0.627-0.841 (Table 4.4). The HPV16 genotype showed a substantial agreement between self-collected and clinician-collected samples (89.2%, k=0.735, Table 4.4), while HPV35 showed an almost perfect agreement between self-collected and clinician-collected specimens (95.4%, k=0.841).

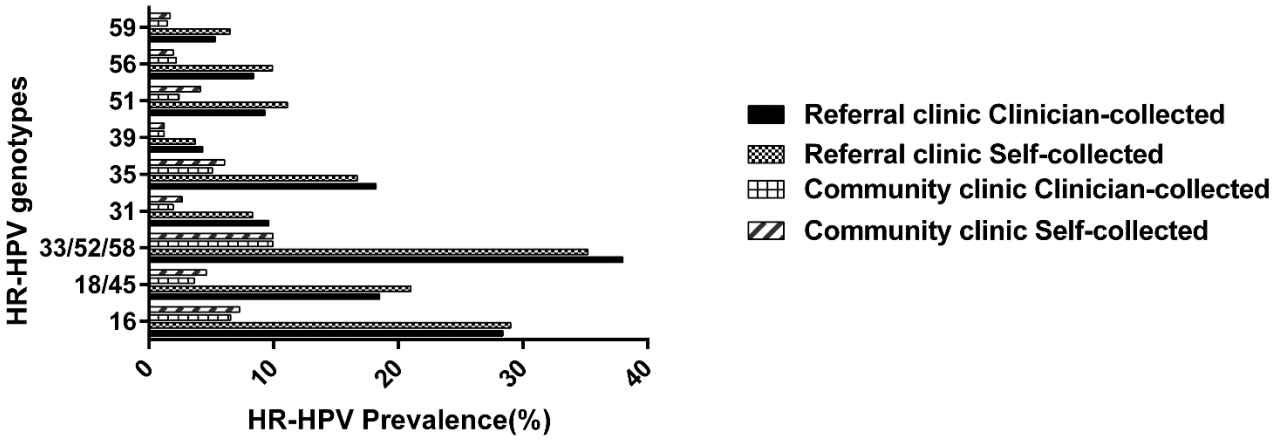

Figure 4.1: Prevalence of HR-HPV genotypes in self-collected and clinician-collected specimens using the hpVIR assay in the community and the referral clinics.

Table 4. 3: Comparison of self-collected and clinician-collected samples from women with single HR-HPV infections and multiple HR-HPV infections from the community and the referral clinics.

	Community clinic			Referral clinic		
Variables	**Clinician-collected**	**Self-collected**	**p-value**	**Clinician-collected**	**Self-collected**	***p*-value**
Single infection	20.4% (85/413)	19.1% (79/413)	0.601	46.0% (149/324)	42.3% (137/324)	0.342
Multiple infection	5.8% (24/413)	8.7% (36/413)	0.108	37.7% (122/324)	37.7% (122/324)	1.000

Table 4. 4: Concordance for HR-HPV genotypes between self-collected and clinician-collected specimen in the community clinic and the referral clinics.

	Community clinic			Referral clinic		
HR-HPV types	**% Agreement**	**kappa value**	**95% CI**	**% Agreement**	**Kappa value**	**95% CI**
16	96.4	0.717	0.581-0.853	89.2	0.735	0.653-0.817
18/45	96.6	0.571	0.369-0.773	90.7	0.705	0.606-0.803
33/52/58	94.0	0.658	0.533-0.782	86.1	0.701	0.670-0.782
31	99.3	0.838	0.659-1.000	96.3	0.780	0.659-0.900
35	96.6	0.678	0.519-0.836	95.4	0.841	0.763-0.919
39	99.8	0.888	0.669-1.000	96.9	0.627	0.413-0.840
51	97.8	0.629	0.406-0.853	93.5	0.660	0.525-0795
56	98.8	0.700	0.449-0.950	96.6	0.801	0.687-0.915
59	99.0	0.709	0.438-0.981	97.5	0.777	0.627-0.926

HR-HPV: high-risk human papillomavirus, **CI**: confidence intervals

4.3.4 HR-HPV genotypes according to histology using *hpVIR* assay

Of the 324 women, histology results were available for 291 women including 2.1% (6/291) with inconclusive results (Table 4.5). More than half of the women had CIN3 (51.2%, 149/291), followed by CIN2 (23.0%, 67/291), no atypia (15.5%, 45/291), CIN1 (4.1%, 12/291) and cervical cancer (4.1%, 12/291). Overall, there was no significant difference in HR-HPV prevalence based on histology between self-collected and clinician-collected samples [78.4% (228/291) vs 81.8% (238/291), p=0.299]. HR-HPV vaccine types (HPV16/18/31/33/45/52/58) were identified in 64.4% vs 46.7% of no atypia, 41.7% vs 41.7% of CIN1, 65.7% vs 59.7% of CIN2, 71.8% vs 71.1% of CIN3 and 75.0% vs 66.7% of cervical cancer between clinician-collected and self-collected, respectively (Table 4.5). The detection rate of the most commonly detected HPV genotypes (HPV16 and -35) was similar between self-collected and clinician-collected samples from women with CIN2/3 and cervical cancer [29.0% (66/228) vs 28.5% (65/228) and 19.7% (45/228) vs 19,3% (44/228), respectively]. Three women with CIN3 tested positive for HPV16 in their self-collected samples and were infected with multiple HPV types but were negative in their clinician-collected samples. The HR-HPV copy number for three self-collected CIN3 samples were above the cut-off for HR-HPV positivity, but two of the samples had a low viral load.

Table 4.5: Distribution of HR-HPV types between self-collected and clinician-collected specimen according to histology results in women from referral clinic.

	Histology results									
	NILM (n=45)		CIN1 (n=12)		CIN2 (n=67)		CIN3 (n=149)		Cervical cancer (n=12)	
HR-HPV types	Clinician-collected	Self-collected	Clinician-collected	Self-collected	Clinician-collected	Self-collected	Clinician-collected	Self-collected	Clinician-collected	Self-collected
16	11	10	0	2	18	17	41	44	6	5
18/45	7	8	1	1	16	19	23	29	3	3
33/52/58	12	9	2	3	19	19	66	62	3	2
31	5	3	2	2	4	6	18	15	0	0
35	6	6	2	1	14	15	29	28	1	2
39	2	0	1	0	2	2	5	8	0	0
51	1	4	1	1	4	7	17	16	1	1
56	4	3	1	1	5	7	12	14	2	2
59	4	3	0	0	4	4	6	13	1	1
HPV multiple infection	14	12	3	4	19	22	61	65	5	4
HR-HPV16/18/31/33/45/52/58	29	21	5	5	44	40	107	106	9	8
HR-HPV negative	13	17	5	5	14	15	20	25	1	1
HR-HPV positive	32	28	7	7	53	52	129	124	11	11

NILM: negative for intraepithelial lesions or malignancy, **CIN**: cervical intraepithelial neoplasia, **HR-HPV**: high-risk human papillomavirus

4.3.5 Acceptance of self-collection of specimens for HPV testing

The participants from both study sites gave a similar response regarding self-collection versus clinician-collection of specimen (Table 4.6). More than half of the women find self-collection and clinician-collection to be interesting (64.4% vs 66.1% in the community-based clinic and 54.0% vs 59.3% in referral clinic, respectively). A very small proportion of women from the community-based clinic experienced discomfort when collecting the sample, both using self-collection (3.4%) and clinician-collection (3.9%). In the referral clinic, a slightly higher proportion of women experienced discomfort with clinician-collection (9.5%) as compared to self-collection (4.3%) of samples. At both study sites older women reported to be embarrassed and were more likely to have had only one Pap smear screening test in their lifetime [73.3% (22/30) self-collected vs 66.7% (20/30) clinician-collected at community-based clinic and 66.1% (39/59) self-collected vs 67.9% (36/53) clinician-collected at referral clinic]. Most participants at both study sites reported that they would be willing to perform self-collection at home and return the card for testing [77.2% in the community-based clinic and 83.0% in referral clinic, (Table 4.7)]. Given a choice, almost all participants preferred clinician-collection to self-collection of samples (95.6% in community-based clinic and 90.7% in referral clinic, Table 4.7).

Table 4.6: Experience with self-collection and clinician-collection.

	Community clinic		Referral clinic	
Variable	**Clinician-collected %(n/N)**	**Self-collected %(n/N)**	**Clinician-collected %(n/N)**	**Self-collected %(n/N)**
Embarrassed	12.8% (53/413)	14.0% (58/413)	16.4% (53/324)	18.2% (59/324)
Self-confident	17.0% (70/413)	16.0% (66/413)	16.7% (54/324)	16.7% (54/324)
Discomfort	3.9% (16/413)	3.4% (14/413)	9.5% (31/324)	4.3% (14/324)
Interested	64.4% (266/413)	66.1% (273/413)	54.0% (175/324)	59.3% (192/324)

n: number of women responded, **N**: total number of study participants

Table 4.7: Investigation of the acceptability of self-collection for HPV testing.

	Community clinic	Referral clinic
Variables	% (n/N)	% (n/N)
Would you be willing to collect a sample yourself (self-sample) at home and bring it in for testing?		
Yes	77.2% (319/413)	83.0% (269/324)
Not sure	18.6% (77/413)	10.8% (35/324)
No	1.9% (8/413)	4.3% (14/324)
If no, why not?		
I prefer the specimen to be taken by the nurse	12.5% (1/8)	12.5% (1/14)
I will not be able to take the specimen correctly	62.5% (5/8)	62.5% (5/14)
Which method do you prefer for cervical cancer screening		
To take a sample myself	1.4% (6/413)	3.7% (12/324)
For a healthcare worker to take a sample	95.6% (395/413)	90.7% (294/324)
Either for myself to take a sample or a health worker to take a sample	1.2% (5/413)	2.5% (8/324)

n: number of women responded, **N**: total number of study participants

4.3.6 HPV detection in cervical clinician-collected specimen using the *hpVIR* assay and HC-2

A total of 628 women were screened for HR-HPV infection using HC-2 and the *hpVIR* assay. Overall, the HR-HPV prevalence was 46.2% using *hpVIR* compared to 48.3% using HC-2, with an agreement of 87.7% (k=0.754, 95%CI;0.703-0.806) between the two assays (Table 4.8).

Table 4.8: Concordance of HR-HPV infection between the *hpVIR* and the HC-2 assays.

		hpVIR			
		HR-HPV positive (%)	**HR-HPV negative (%)**	**Total (%)**	**% Agreement**
HC-2	**HR-HPV positive (%)**	258 (41.1)	45 (7.1)	303 (48.3)	87.7 (k=0.754)
	HR-HPV negative (%)	32 (5.1)	293 (46.7)	325 (51.7)	
	Total (%)	290 (46.2)	338 (53.8)	628 (100.0)	

HR-HPV: high-risk human papillomavirus, **k**-kappa value

4.4 DISCUSSION

The present study assessed the prevalence of HR-HPV genotypes between self-collected and clinician-collected samples applied to FTA cards in women from a community-based clinic and a referral clinic in Eastern Cape, South Africa. We observed high HR-HPV prevalence, overall

agreement of HR-HPV infection, and a similar distribution of HR-HPV genotypes between self-collected and clinician-collected samples. A similar trend has been reported using FTA cards and PCR-based assays performed in women from the general population and referral population (Guan et al. 2013; Geraets et al. 2013; Dijkstra et al. 2012). Our findings confirm that the self-collection method and storage on FTA cards is an adequate procedure for HPV DNA testing and support the use of self-collection as an alternative strategy in the regular cervical cancer screening routine. Many developed countries have transitioned to HPV DNA testing, and cervical cancer screening guidelines with HPV DNA testing has been implemented (Committee 2014; Polmanet al. 2019; Arbyn et al. 2015). However, this is the opposite for low-income countries having low participation rate in cervical cancer screening particularly in rural regions. Therefore, introducing new screening strategies for women experiencing screening barriers will improve uptake and could have an impact on the incidence of cervical cancer diseases (Gustavsson et al. 2018).

The prevalence of HR-HPV infection and distribution of HR-HPV genotypes differs between regions and countries (Bruni et al. 2019a; de Sanjosé et al. 2007). In the current study, HPV16 and HPV35 were the most frequently detected genotypes in both self-collected and clinician-collected samples in the two sites. Other cross-sectional studies conducted in South Africa also reported HPV35 and HPV16 as the most commonly identified genotypes among women with or without cervical abnormalities (Dols et al. 2012; McDonald et al. 2012; McDonald et al. 2014). However, the predominant HR-HPV genotypes in other African studies were HPV18 & HPV59 in Ghana and HPV52 & HPV16 in Tanzania (Dols et al. 2012; Awua et al. 2016). A recent study done in Brazil among women residing in rural areas showed HPV56 and HPV51 the common types in their population (Lorenzi et al. 2019). The two HR-HPV genotypes (HPV16 and 35) are among the top five causing cervical cancer in South Africa (Bruni et al. 2019b). None of the HPV vaccines protect against HPV35 (overall prevalence =18.5%, mostly in multiple infections); an important HR-HPV genotype in women with cervical disease in the Eastern Cape. A high proportion of 72.0% CIN3/cervical cancer women were more likely to harbour vaccine HR-HPV types (HPV16/18/31/33/45/52/58). Our results indicate that studies on the distribution of HPV genotypes in cervical cancer not present in the current vaccines

should be considered when developing strategies and targets for the next generation of HPV vaccines.

A slightly higher prevalence of multiple HR-HPV infections was observed in self-collected as compared to clinician-collected samples in the community-based clinic which could reflect the anatomic area sampled. Vaginal self-collected samples contain vaginal fluid with both cervical and vaginal cells. Women are more likely to be infected with several HPV genotypes in the vagina that are not present in the endocervix (Gustavsson et al. 2011; Dijkstra et al. 2012). HR-HPV genotypes at low viral load present only in vaginal self-collected samples are not correlated with cervical cancer diseases (Gustavsson et al. 2011; Zhang et al. 2014). The few cases of discordance of HPV16 between self-collected and clinician-collected samples among women with CIN3 in our study, were likely to be due to low viral load.

There was high concordance in HR-HPV detection between self-collected and clinician-collected samples in the present study. The use of the Viba-brush in combination with FTA cards, yields good quality DNA in both self-collected and clinician-collected specimen, indicating that the self-collection was performed correctly. Sampling devices for HPV DNA testing have a strong effect on the detection of HPV infection. Self-sampling with a brush-based sampling device in combination with FTA cards was found to be acceptable among women and this together with the clinically validated PCR-based HPV DNA test yielded high agreement in identifying women with high risk of CIN2+ in comparison with clinician-collection sampling (Dijkstra et al. 2012). The in-house *hpVIR* assay combined with the FTA cards performed well compared to HC-2, as demonstrated by substantial agreement in HR-HPV positivity. Our findings concur with previous studies reporting on the performance of the two assays, indicating that the *hpVIR* assay and FTA cards can be utilised for primary screening programmes in regions other than Sweden (Gustavsson et al. 2019; Gustavsson et al. 2009).

At both study sites, women showed the same attitude regarding self-collection versus clinician collection samples for HPV DNA testing. The high rate of willingness to perform self-collection was not influenced by sociodemographic factors or any other factor studied as observed in previous studies (Murchland et al. 2019; Morgan et al. 2019). This underscores

the strong scientific basis for implementing self-collection in cervical cancer screening, thereby enabling women from communities and rural settings where cervical cancer screening programmes are presently not available to participate. Moreover, the high rate of acceptability for vaginal self-collection could influence the effectiveness of HPV self-collection in routine screening programme (Polmanet al. 2019). Although self-collection was highly accepted, the majority of women preferred the specimen to be taken by a healthcare worker. In our study, we did not investigate factors influencing women's choice as to why they prefer clinician-collection of samples. Mao and colleagues reported that women who prefer a clinician-collection want to have a one-on-one consultation with a clinician, so that they can address other issues they may have (Mao et al. 2017). Other factors that women are raising include adequacy of the specimen and lack of confidence to perform self-collection correctly (Morgan et al. 2019; Saidu et al. 2018). Therefore, our findings suggest that women should be given an opportunity to choose which method they prefer for specimen collection, when participating in the cervical cancer screening programme.

A large population of women in the Eastern Cape Province reside in rural areas and are less likely to participate in cervical cancer screening programme because of the lack of nearby facilities with functional cervical cancer screening programmes (Hoque et al. 2008). Implementing self-sampling in this region will therefore have an impact on the screening programme since it does not require infrastructure. This approach allows women who are unwilling to attend Pap smear test performed by clinician to have access to cervical screening and give them privacy to collect the specimen (Guan et al. 2013). Implementation of self-collection is also more likely to create awareness and more information about HPV infection than clinician-collection (Allen-Leigh et al. 2017; Crofts et al. 2015). There are different methods to administer the self-collection kit, including mailing the kit directly to the woman, conducting community-based campaigns and using an opt-in strategy (Arbyn et al. 2018). In developed countries, such as Sweden, mailing self-collection kits to underscreened women has been shown to work effectively (Gustavsson et al. 2018). However, in South Africa, it will be challenging to send self-collection kits as the postal system is not working effectively or does not exist in some rural areas. Direct offer of self-collection kit would be a better method to achieve knowledge and confidence among women residing in rural communities as this

method has been previously shown to have significantly high rate (>75%) among underscreened women (Arbyn et al. 2018). A second issue would be loss of women to follow-up due to difficulty in travelling to the clinics, something which would have a strong negative impact on the efficiency of cervical cancer screening programmes. Therefore, effective communication strategies to follow-up women with HR-HPV positive test needs to be developed prior to implementation of self-collection for HPV testing outside the clinics in South Africa.

The FTA cards provide several advantages for self-collection in this environment. These strategies have the potential to improve cervical cancer screening programmes by increasing the screening coverage, reducing the work load for the clinicians, and identify women at high-risk of developing cervical cancer (Gustavsson et al. 2018; Arbyn, Smith, et al. 2018). Furthermore, this allows healthcare facilities with limited resources for cervical cancer screening to target women at high-risk of cervical cancer. However, self-collection for HPV has been found to increase the rate of women being referred to colposcopy clinics and for treatment. Biomarkers such as DNA methylation has been reported to triage self-collected HR-HPV positive women, but further studies are needed to determine its clinical value (Snellenberg et al. 2012). Repeating the HPV test in 4-6 months for women that are HPV-positive in their screening test, can be used to identify women with persistent infection and thereby increase the sensitivity and specificity of screening (Gustavsson et al. 2018). If it was possible to rescreen women, this may be the best approach to triage women for referral to the treatment clinic.

A potential limitation in this study could be the use of in-house *hpVIR* real time PCR assay. The *hpVIR* assay is clinically validated HPV test that gives HPV type information, but it does not distinguish between the alpha HPV types, HPV18/45, and HPV33/52/58 because they are closely related. This limits the ability to provide the prevalence of the individual HPV types that are grouped in the *hpVIR* assay.

4.5 CONCLUSION

The study shows a high concordance in HR-HPV genotype prevalence between self-collected and clinician-collected specimens. The frequency distribution of HR-HPV genotypes will assist in identifying and monitoring genotypes that are not covered by the vaccines currently in use. The self-collection procedure for HPV testing was regarded as highly acceptable by the women. Self-collection can have a positive impact on the cervical screening programme in South Africa by increasing the population coverage in rural areas and enable women who are unable to attend clinics to participate in cervical cancer screening.

CHAPTER 5: Detection of Sexually Transmitted Pathogens and Co-Infection with Human Papillomavirus in Women Residing in Rural Eastern Cape, South Africa

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ABSTRACT

Background: South African women of reproductive age have a high burden of sexually transmitted infections (STIs), including human papillomavirus (HPV) infection. However, there is limited information on the prevalence of sexually transmitted pathogens in women from rural Eastern Cape Province, South Africa. The study aims at determining the prevalence of sexually transmitted pathogens and co-infection with high-risk (HR) HPV among women from rural Eastern Cape Province, South Africa.

Method: A total of 205 cervical specimens were collected from women aged ≥30 years from a rural community-based clinic. The samples were tested for a panel of pathogenic STIs [*Chlamydia trachomatis* (serovars A-K & L1-L3), *Haemophilus ducreyi*, Herpes Simplex Virus (Types 1 & 2), *Neisseria gonorrhoeae (NG)*, *Treponema pallidum*, *Trichomonas vaginalis* (*TV*), and pathobionts [*Mycoplasma genitalium* (*MG*)*, Mycoplasma hominis* (*MH*) and *Ureaplasma* spp. (*UP*)] using a multiplex PCR STD direct flow chip assay through a manual Hybrispot platform (Master Diagnostica, Granada, Spain). HR-HPV detection was performed by HC-2 assay.

Results: High-risk HPV prevalence was 32.2% (66/205) and HIV-1 prevalence was 38.5% (79/205). The overall prevalence of six pathogenic STIs was 22.9% (47/205), with *TV* having the highest prevalence (15.6%; 32/205). UP (70.2%, 144/205) and *MH* (36.6%, 75/205) were the most frequently detected pathobionts. Co-infection with ≥2 pathogens / pathobionts was observed among 52.7% (108/205) participants. Of the six pathogenic STIs, three participants had more than one STI (1.46%) with the presence of *MH* and *UP*. HSV-2 (OR: 4.17, 95% CI: 1.184-14.690) and HIV infection (OR: 2.11, 95% CI: 1.145-3.873) were independent STIs associated with HR-HPV infection.

Conclusions: The high prevalence of pathogenic STIs underscores the need to improve syndromic management policy by implementing effective strategies of prevention, screening tests, and management. HSV-2 and HIV-positive remain strongly associated with HR-HPV infection.

5.1 INTRODUCTION

Internationally, sexually transmitted infections (STIs) are a significant public health problem, with approximately more than one million people infected each day. According to the World Health Organization, the global estimate of new infections with commonly treatable STIs [*Trichomonas vaginalis (TV), Treponema pallidum* (*TP*)*, Chlamydia trachomatis* (*CT*) and *Neisseria gonorrhoeae* (*NG*)] was 376.4 million in 2016 (WHO 2018). Africa accounts for 69 million new infections of treatable STIs with women having a high burden of *TV* (11, 8%) and *CT* (5, 0%) (WHO 2018). STIs have an impact on women's health, associated with cervicitis, urethritis, pelvic inflammation, complications of reproductive health, and poor pregnancy outcomes (Mermelstein and Plax 2016). South African women have a high prevalence of STIs, ranging from 12.7% to 47.8%, which differs by age, region, and population (Moodley et al. 2015; Joseph Davey et al. 2019; Mudau et al. 2018; Naidoo et al. 2014). The burden of STIs is more common among women of reproductive age (15-49 years) (WHO 2018). The acquisition of new STIs occurs at all ages, and high rates of STIs are more likely to be found in younger women (Naidoo et al. 2014). In South African studies, women age <25 years are almost 2-fold more likely to have STIs compared to older women (>25 years) and have high rates of co-infections (Naidoo et al. 2014; Mbulawa et al. 2018; Menezes et al. 2018).

Human papillomavirus (HPV) is the most common and infectious viral STI, with 291 million new infections estimated to have occurred in 2016, with a particularly high burden among women in Southern Africa (Bruni et al. 2010; WHO 2018). Most HPV infections are transient, but 5% remain persistent and can progress to high-grade lesions or cervical cancer (Schiffman et al. 2011). The high burden of HPV infection is influenced by several factors, including co-infection with other STIs. HIV infection is a significant independent factor of HPV, and infection with either HPV or HIV is thought to enhance the spread of the other infection (Smith-McCune et al. 2010). HIV-positive women have been reported to have a higher prevalence and higher viral load of high-risk (HR) HPV compared to HIV-negative women (Taku et al. 2020). In addition, *CT*, Herpes Simplex Virus-2 (HSV-2), and *NG* increase the risk of HIV acquisition (Adachi et al. 2015; Johnson and Lewis 2008; Looker et al. 2017). These STIs are co-factors of HPV and may have an impact on the natural history of HPV (Deluca et al. 2011; de Abreu et al. 2016; Paba et al. 2008; Smith, Herrero, et al. 2002; Smith, Muñoz, et al.

2002). The association of HPV infection with some of these STIs is due to chronic inflammation or immunosuppression, which promotes the susceptibility to and progression of HR-HPV persistent infection (Adefuye and Sales 2012; Denny et al. 2012; Liu et al. 2016; Paba et al. 2008; Silins et al. 2005). HPV-positive women co-infected with one or more of these STIs are at high risk of developing cervical cancer diseases and invasive cervical cancer (Smithet al. 2002; Paba et al. 2008; Deluca et al. 2011). Consequently, women harbouring or having a history of *CT* are less likely to clear HPV infection, and two times more likely to develop cervical cancer diseases (Lehtinen et al. 2011; Jensen et al. 2014; Vriend et al. 2015). Moreover, sexually transmitted pathobionts such as *Mycoplasma hominis* (*MH*) and *Ureaplasma* spp. (*UP*) are associated with an increased risk of HPV persistent infection and abnormal cervical cytology (Parthenis et al. 2018). Therefore, it is crucial to screen for these sexually transmitted pathogens to reduce the risk of transmission and their outcome.

In South Africa, the current strategy for diagnosing STIs is through clinical indicators such as vaginal discharge, pelvic pain, and ulcerative genital lesions (Department of National Health 2015). The syndromic management approach has been successful in treating many pathogens causing STIs and reducing the burden of other STIs such as *TP* (Kularatne et al. 2018). However, this approach may result in overtreatment, antimicrobial resistance, and is not effective in people with STIs who do not show any clinical symptoms (Mayaud and Mabey 2004). South African studies reported a high prevalence of asymptomatic women, ranging from 50-75%, harbouring genital tract infections (Francis et al. 2018; Wilkinson et al. 1999). Since many STIs are asymptomatic and missed by the syndromic management approach, laboratory-based diagnosis remains the only strategy that allows the detection of genital tract infections (Sznitman et al. 2010).

Moreover, screening programmes allow people to be informed about STIs, which helps to prevent and manage the spread of STIs (Sznitman et al. 2010). Women residing in rural areas have limited knowledge of STIs and not likely to be informed about STIs services due to lack of access to healthcare facilities or facilities having limited resources to treat STIs (Cristillo et al. 2017; Wi et al. 2019). There is limited information on STIs and the prevalence of specific sexually transmitted pathogens in Eastern Cape Province. Therefore, the study aims at

investigating the prevalence of sexually transmitted pathogens in women from rural Eastern Cape using molecular detection.

5.2 MATERIALS AND METHODS

Cohort description or description of study participants: Two hundred and five cervical samples were selected from a cross-sectional study done between September 2017 and August 2018. The cross-sectional study has been described in detail previously (Taku et al. 2020b). Briefly, women aged 30 years or more attending cervical cancer screenings or for other reasons were recruited from a community-based clinic within the OR Tambo District, Eastern Cape. All signed consent forms were obtained from all enrolled women. Women were requested to test for HIV if they were not aware of their HIV status or if their HIV status was not documented on their health card. Women received pre-HIV testing counselling prior to and after testing them for HIV using a rapid test (Alere Determine^{TM} HIV-1/2 Ag/Ab Combo, Alere, Waltham, MA).The protocol of this study was approved by the Human Research Ethics Committees of the University of Cape Town (UCT) (HREC reference 615/2017), Walter Sisulu University (reference 090/2016), and Eastern Cape Department of Health Ethics (EC reference 2017RP0_484). Cervical specimens were stored in the Digene Specimen Transport Medium (Qiagen Inc., Gaithersburg, MD; USA), transported to UCT, and kept at -80^{O}C until further analysis.

There was no special selection criteria considered for this study. The median age of the women was 45 years (IQR: 38-53), and the median number of lifetime sexual partners was three. A total of 61.5% (126/205) of the women reported not using a condom during their last sexual encounter. More than half of women (58.5%, 120/205) reported not using any method of contraception with their current partner. One hundred and ten (110) study participants (53.6%) reported having had vaginal discharge with 44.5% (49/110) reporting to have occurred more or equal to six months (Table 5.1). A proportion of 38.5% (79/205) women were positive for HIV and 96.2% (76/79) were on antiretroviral drugs. Majority of women on cytology test were negative for intraepithelial lesions (88.8%, 182/205), 7.8% (16/205) for ASCUS, 2.0% (4/205) for low grade intraepithelial squamous lesions and 1.0% (2/205) for high

grade squamous intraepithelial lesions. One participant had had inadequate result (0.5%,1/205).

Table 5.1: Description of the study participants.

Variables	% (n/N)
Age in years, median (IQR)	45 (38-53)
Age category	
30-39 years	33.7% (69/205)
40-49 years	28.8% (59/205)
≥50 years	37.6% (77/205)
Lifetime partners	
1	15.1% (31/205)
2	29.8% (61/205)
≥3	55.1% (113/205)
Sexual partners past 12 months	
0	26.3% (54/205)
≥1	73.7% (151/205)
Sexual partners past 1 month	
0	41.5% (85/205)
≥1	58.5% (120/205)
Frequency of vaginal sex past 1 month	
0	49.3% (101/205)
1-3	33.2% (68/205)
≥4	17.1% (35/205)
Used condoms during last sexual intercourse	
No	61.5% (126/205)
Yes	37.1% (76/205)
Discharge	
No	46.3% (95/205)
Yes	53.7% (110/205)
Frequency of vaginal discharge	
Current/last week	18.1% (37/205)
More than a week and less than 6 months	11.2% (23/205)
More than or equal to 6 months	23.9% (49/205)
Using any contraception with current partner	
No	58.5% (120/205)
Yes	40.0% (82/205)
Pregnancy	
No	4.4% (9/205)
Yes	95.6% (196/205)
HIV infection	
Negative	61.5% (126/205)
Positive	38.5% (79/205)

n: number of women responded, **N**: total number of study participants

5.2.1 DNA extraction

The DNA was extracted from each cervical specimen (400µl) using the MagNA Pure Compact Nucleic Acid Isolation kit (Roche diagnostic, Mannheim, Germany) on an automated Roche MagNA Pure Compact system. DNA was eluted in 100µl elution buffer and stored at -20^{o}C until further use.

5.2.2 Detection of sexually transmitted pathogens

Extracted DNA was used for detection of STD performed using multiplex PCR STD direct flow chip assay through a manual Hybrispot platform (Master Diagnostica, Granada, Spain)

following the manufacturer's instructions. The panel detects the following pathogens: *TP*, HSV (Type 1&2), *TV*, *CT* (Biovar LGV: Serovars L1-L3 & Serovars A-K), *NG*, *Haemophilus ducreyi* as well as *UP* (urealyticum/parvum), *MG* and *MH* (Barrientos-Durán et al. 2020). The image results of each chip membrane were captured by a camera, and analysis was performed automatically with HybriSoft software.

5.2.3 Detection of HR-HPV infection

Cervical specimens in Digene transport medium were tested for 13 HR-HPV types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) using the Hybrid Capture-2 (HC-2) assay (Qiagen Inc., Gaithersburg, MD; USA) according to the manufacturer's protocol. A ratio of relative light units/cut-off ≥1 was considered positive, while a ratio of <1 was considered negative for HR-HPV types.

5.2.4 Statistical analysis

Single infection was defined as being positive for one of any pathogenic STIs or pathobionts. Multiple infections was defined as having two or more microorganisms (pathogenic STIs/pathobionts). Statistical analysis was performed using STATA 15.0 (STATA Corp, College Station, TX, USA). Univariate logistic regression models were conducted to determine the association between sexually transmitted pathogens and HR-HPV infection. Multivariate analysis was done using the statistically significant variables (p-value<0.05) of the univariate logistic regression models to identify the sexually transmitted pathogens that are independently associated with HR-HPV infection.

5.3 RESULTS

5.3.1 Sexually transmitted pathogens / pathobionts and pattern of infection

HR-HPV prevalence was 32.2% (66/205) and HIV-1 prevalence was 38.5% (79/205). The overall prevalence of the six STIs was 22.9% (47/205) with a highest number of women positive for *TV* (15.6%, 32/205) followed by HSV-2 (5.9%, 12/205), *CT* (2.4%, 5/205) and *NG* (1.5%, 3/205) (Table 5.2). Of the pathobionts, *UP* (70.2%, 144/205) and *MH* (36.6%, 75/205) were the most frequently detected (Table 5.2). Overall, the prevalence of single infection was 31.2% (64/205), with most women infected with *UP* (62.5%) and HR-HPV infection (17.2%)

(Figure 5.1 A, B and C). Furthermore, multiple infections were found in 52.7% (108/205) women, with 30.7% had dual infection (2 pathogens or pathobionts) and 22.0% co-infected with more than three pathogens or pathobionts (Figure 5.1 B, C & D). A higher proportion of co-infection was observed among women with *UP,* whereby *UP*/*MH* (26.9%), *UP*/HPV (21.3%), and *MH*/*UP*/TV (10.2%) were the most commonly detected co-infections (Figure 5.1 C and D). Of the six pathogenic STIs, three participants had more than one STI (1.46%) with the presence of *MH* and *UP*. Women with HSV-2 infection were almost five times more likely to be infected with HR-HPV (OR: 4.65, 95% CI: 1.35-16.071). In the multivariate analysis, HSV-2 (OR: 4.17, 95% CI: 1.184-14.690) and HIV infection (OR: 2.11, 95% CI: 1.145-3.873) remained the significant risk factors of HR-HPV infection (Table 5.3).

Table 5.2: Prevalence of sexually transmitted infections detected using multiplex PCR STD direct flow chip assay.

Variables	% (n/N)
Chlamydia trachomatis (serovars L1-L3)	0.5% (1/205)
Chlamydia trachomatis (serotypes A-K)	2.0% (4/205)
Herpes simplex virus Types I	0.0% (0/205)
Herpes simplex virus Types II	5.9% (12/205)
Trichomonas vaginalis	15.6% (32/205)
Neisseria gonorrhoeae	1.5% (3/205)
Treponema pallidum	0.0% (0/205)
Haemophilus ducreyi	0.0% (0/205)
Mycoplasma hominis	36.6% (75/205)
Ureaplasmas (U. urealyticum or U. parvum)	70.2% (144/205)
Mycoplasma genitalium	1.5% (3/205)

n: number of women responded**, N:** total number of study participants.

Table 5.3: Association of sexually transmitted pathogens and HR-HPV infection.

	HR-HPV prevalence	Univariate analysis		Multivariate analysis	
Variables	**% (n/N)**	**OR (95% CI)**	**P-value**	**OR (95% CI)**	***p*-value**
Mycoplasma hominis					
Negative	33.9% (44/130)	ref			
Positive	29.3% (22/75)	0.81 (0.438-1.502)	0.506		
Ureaplasmas species (U. urealyticum or U. parvum)					
Negative	24.6% (15/61)	ref			
Positive	35.4% (51/144)	1.68 (0.856-3.305)	0.131		
Trichomonas Vaginalis					
Negative	31.8% (55/173)	ref			
Positive	34.4% (11/32)	1.12 (0.507-2.493)	0.838		
Herpes Simplex Virus Types II					
Negative	30.1% (58/193)	ref		ref	
Positive	66.7% (8/12)	4.65 (1.35-16.071)	**0.015**	4.17 (1.184-14.690)	**0.026**
Chlamydia Trachomatis (serovars L1-L3 & A-K)					
Negative	32.5% (65/200)	ref			
Positive	20.0% (1/5)	0.52 (0.057-4.739)	0.561		
Neisseria gonorrhoeae					
Negative	32.2% (65/202)	ref			
Positive	33.3% (1/3)	1.05 (0.094-11.834)	0.966		
Mycoplasma genitalium					
Negative	31.7% (64/202)	ref			
Positive	66.7% (2/3)	4.31 (0.384-48.434)	0.236		
HIV infection					
Negative	25.4% (32/126)	ref		ref	
Positive	43.0% (34/79)	2.22 (1.219-4.042)	**0.009**	2.11 (1.145-3.873)	**0.017**

HR-HPV: high-risk human papillomavirus, **OR**: odds ratio, **CI**: confidence intervals, **ref**: reference**, Highlighted values**: significant p-value, **ref**: reference; **n**: number of women responded, **N**: total number of study participants.

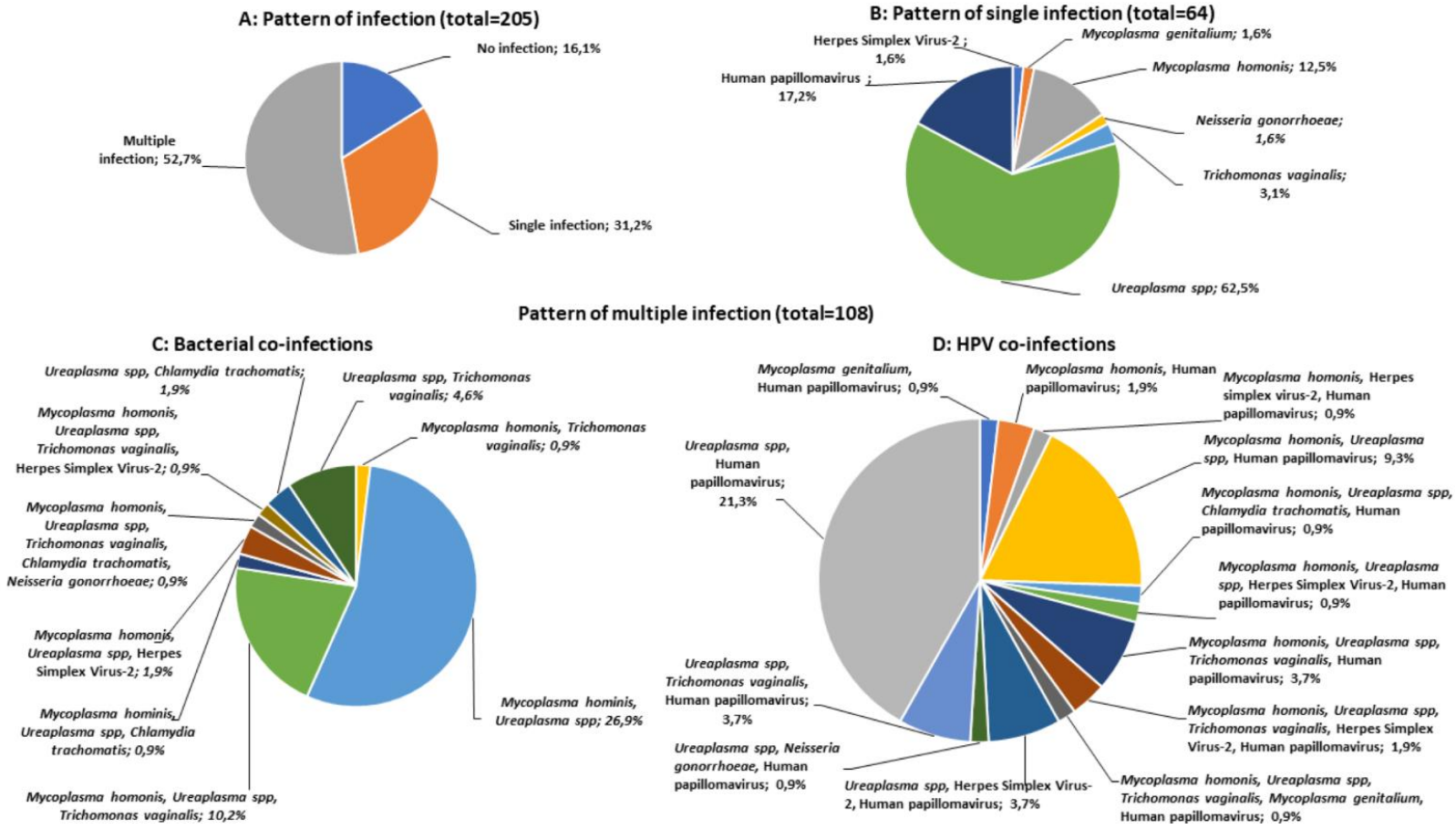

Figure 5.1: Patterns of sexually transmitted pathogens in women of rural South Africa. A) The pattern of infections (no infection, single infection, and multiple infections) B) The pattern of single infections in women with one of the nine sexually transmitted pathogens [*Chlamydia trachomatis* (*CT*), *Herpes simplex virus-2* (HSV-2), *Mycoplasma genitalium* (*MG*), *Mycoplasma hominis* (*MH*), *Neisseria gonorrhoeae* (*NG*), *Trichomonas vaginalis* (*TV*), *Ureaplasma* spp. (*UP*), and Human papillomavirus (HPV)]. C) The pattern of HPV co-infections and bacterial coinfection in women with more than one sexually transmitted pathogens evaluated.

Of the women with self-reported vaginal discharge, a total of 21 (19.1%) were positive for treatable STIs, including 56.3% (18/32) with *TV* and 60.0% (3/5) with *CT*. All women positive for *NG* self-reported not having had vaginal discharge (100.0%, 3/3). Similarly, the pelvic examination done by the study nurse showed that 28 .1% (9/32) of women positive for *TV* had vaginal discharge but none of the women positive for *NG* had vaginal discharge. The association of STIs and behavioural factors are depicted in Table S5.1. Women having 3 or more lifetime sexual partners were associated with STIs (OR: 3.69, 95% CI: 1.047-12.986, *p*=0.042). In addition, women aged ≥50 years (OR:0.38, 95% CI:0.185-0.797, *p*=0.010), three or more lifetime sexual partners (OR:3.08, 95% CI:1.098-8.619, *p*=0.033), HIV positive (OR:2.22, 95% CI:1.219-4.042, P=0.009) and reported having had vaginal discharge more than or equal to six months (OR:0.38, 95% CI:0.149-0.967, *p*=0.042) were significant factors of HR-HPV infection (Table S5.2). However, in the multivariate none of these factors remained the significant risk factor of HR-HPV infection (Table S5.2).

5.4 DISCUSSION

We investigated the prevalence of sexually transmitted pathogens or pathobionts and co-infection with HPV infection among women from rural Eastern Cape. The study demonstrates a high overall prevalence of the conventional pathogenic STIs (22.9%) and confirms the high prevalence of *TV* (15.6%) in this population. The high burden of *TV* has been previously reported in South African women of a rural region, occurring in 66% of asymptomatic women (de Waaij et al. 2017). It has been found that hormonal changes and menstrual bleeding contribute to the increase of *TV* and put women at high risk of being more susceptible to acquisition and persistent infection (Poole and McClelland 2013). Women with *TV* persistence have an increased risk of acquiring HIV, a high viral load of HIV, and a likelihood of transmitting HIV infection to their sexual partners (Van der Pol 2007; de Waaij et al. 2017). Also, the high prevalence of *TV* may cause serious reproductive health problems in this group of women, as shown in previous studies (Kissinger 2015). Therefore, better screening programmes and control measures to reduce the burden of this STI are of critical importance, particularly among asymptomatic women.

The positivity rate for *CT* (2.4%) and *NG* (1.5%) was low in this cohort and similar to that observed in community-based studies conducted among older women from rural and urban regions of sub-Saharan Africa (Dubbink et al. 2018). The highest rates of *CT*/*NG* are usually observed among younger women (<25 years) because of biological vulnerability (such as immature ectopic tissue on the cervix) and sexual behaviour which makes them prone to the growth of these pathogens (Lee, Tobin, and Foley 2006; Menezes et al. 2018). For example, amongst asymptomatic young HIV-negative South African women (<25 years), high rates of 33.5% and 11.1%, have been recorded for *CT* and *NG*, respectively (Menezes et al. 2018). Sexual behaviour was a significant risk factor, suggesting that more campaigns are needed to educate younger women and men about sexual and reproductive health (Menezes et al. 2018).

Notably, a high prevalence of *UP* (70.2%) and *MH* (36.6%) was observed, occurring in multiple infections. *UP* and *MH* are emerging pathobionts found in women both with healthy and unhealthy vaginal microbiota (Cox et al. 2016; Waites, Katz, and Schelonka 2005; Rumyantseva et al. 2019). The prevalence of *UP* is between 40-80%, while *MH* ranges between 21-53% in cervical/vaginal specimens of sexually active women (Cox et al. 2016; Waites, Katz, and Schelonka 2005). Previous studies have reported an association of these pathogens with bacterial vaginosis (BV) and STI, such as *MG*, and *CT* (Marovt et al. 2015). Rumyantseva and colleagues (2019) reported a significantly higher prevalence of *UP* (73.4%) among women with BV compared to women with normal bacterial flora (49.4%) or aerobic vaginitis (28.4%) (Rumyantseva et al. 2019). *MH* is considered BV-associated bacteria and has been reported to have a significantly higher bacterial load in women with BV compared to women without BV (Rumyantseva et al. 2019; Sha et al. 2005). Women with BV are reported to have high vaginal pH (>4.5) which is a favourable vaginal environment for pathogenic organisms (Kaambo et al. 2018). Additionally, women with detectable *MH* were often found to be co-infected with *Gardnerella vaginalis*, and such co-infection has been demonstrated in 60.7% of BV-positive women compared to BV-negative women (8.8%), which demonstrate a possible interaction between these pathogens (Cox et al. 2016). The transmission of either *MH* or *Gardnerella vaginalis* could activate the growth of the other, which may promote or contribute to the progression of BV (Cox et al. 2016). Verteramo and colleagues reported *UP*

and *MH* as opportunistic pathogens of the lower female genital tract (Verteramo et al. 2009). However, The European STI guidelines do not recommend routine screening and treatment for these pathogens (Horner et al. 2018).

In this study, more than half of the study participants harboured multiple infections with sexually transmitted pathogens (52.7%). Multiple infections are reported to have a negative impact on the treatment of STIs and regarded as a risk factor for cervical cancer (Magaña-Contreras et al. 2015). Of the multiple infections, the co-infection of *UP*/HR-HPV occurred at a rate of 21.3% women, similar to that reported among sexually active women attending the outpatient clinic for routine cervical cancer screening (Parthenis et al. 2018). Moreover, a study among reproductive-age women from Gambia reported that 50% of women with HPV infection were co-infected with *UP* (Camara et al. 2018)**.** Women with detectable *UP* are found to have high levels of inflammatory cytokines, a biological co-factor that may increase the probability of persistent HPV infection and development of precancerous lesions (Biernat-Sudolska et al. 2011; Lobão et al. 2017; Roeters et al. 2010). Similarly, the prevalence of *UP* was significantly 2-fold higher in women with high-grade squamous intraepithelial lesions (57.5%) compared to women with normal cytology (21.3%) (Farag et al. 2013). The interaction of HR-HPV with *UP* demonstrates the need to screen for these pathogens as they may play a significant role in initiating the development of cervical cancer lesions.

The significant association of viral STIs (HSV-2 and HIV infection) with HR-HPV infection has been previously observed in other studies (Li and Wen 2017; Taku et al. 2020). HSV-2 increases the odds of acquiring other STIs (such as *NG*) and is considered as the significant co-factor for HPV in the development of cervical cancer (Li and Wen 2017; Smith et al. 2002; Venkatesh et al. 2011). Women positive for HSV-2 and HIV infection were reported to have cervicovaginal inflammation and harbour a high diversity of microbes (Keller et al. 2019). These viral STIs have been found as independent risk factors of cervical cancer diseases. For example, a case-control study showed that HIV-positive women had a significantly increased prevalence of abnormal cytology (13.0%) compared to HIV-negative women (5.0%) (Suehiro et al. 2020). Similarly, the presence of HSV-2 infection was 5-fold higher in women with CIN and squamous cell carcinoma compared to those with normal cervical cytology (Zhao et al.

2012). Moreover, the co-infection of HPV/HSV-2 was significantly associated with cervical cancer lesions and cervical cancer than healthy women suggesting that this co-infection could be involved in the progression of cervical cancer (Zhao et al. 2012). The findings highlight the need to consider awareness and educational programmes about the risk of these viruses in order to help reduce their outcome.

With South Africa having a high burden of STIs, particularly among asymptomatic women, an effective strategy to diagnose and treat STIs is needed. The high prevalence of HPV observed in this population requires the need of HPV vaccination strategies. Syndromic management policy has been reported to have low specificity and sensitivity in identifying the most common STIs, such as *NG* and *CT* (Maina, Kimani, and Anzala 2016; Marx et al. 2010). The syndromic management approach may not be good enough to control STIs when utilised alone as it results in a high STI prevalence of undiagnosed infections that may facilitate the transmission of HIV infection (Ward and Rönn 2010). The high prevalence of STIs in this region encourages the need to implement diagnostic STI screening tests as the potential strategy to effectively decrease the burden of STI since a majority of STI-positive women are asymptomatic and remain untreated (Barnabas et al. 2018). The screening will be beneficial not only for asymptomatic women but for those with symptoms in the general population or high-risk populations and facilitate receipt of appropriate treatment. Furthermore, considering the high prevalence of HR-HPV observed in this population, STI screening would be of assistance as it will help to reduce the burden of sexually transmitted pathogens that could potentially promote the development of persistence and cervical cancer lesions (Yong Il 2017).

We acknowledge that the study had some potential limitations including small sample size and The study population were participants from community clinic and does not represent the population of rural Eastern Cape Province of South Africa; therefore, the findings cannot be used of this study cannot be regarded as the representative sample for the whole population of rural Eastern Cape Province. Also, the study was designed for HPV screening and this may result to a potential sampling bias in the context of sexual transmitted infection.

Furthermore, in this study we depended on self-reported questionnaire for some data such as vaginal discharge, frequency of vaginal discharge and sexual behaviour. Therefore, this information may also introduce bias during the collection of participant information and analysis.

5.5 CONCLUSION

A high prevalence of sexually transmitted pathogens, particularly *TV*, *UP*, and *MH* was documented in this rural community. HSV-2 and HIV were co-factors strongly associated with HR-HPV infection. The high prevalence of these pathogens underscores the need to revise the syndromic management policy by implementing effective strategies of prevention, screening tests, and management for sexually transmitted pathogens. The study also highlights the need to encourage routine screening of STIs for all women screened for cervical cancer.

CHAPTER 6: CONCLUSIONS

The burden of cervical cancer has been reduced in developed countries due to the implementation of improved cervical cancer screening programs and HPV vaccination, but it remains a public health problem in low- and middle-income countries such as South African. It is important to note that the participants included in this study were from a rural community clinic and referral gynaecology outpatient clinic. This is the first study to investigate HPV prevalence, risk factors for HPV infection and comparing cervical cancer screening methods in women from the Eastern Cape of South Africa. Moreover, this is the first study to determine the distribution of HR-HPV genotypes in women with or without cervical intraepithelial lesions from this community. This thesis provides insight on HPV infection, potential risk factors for HPV infection and cervical cancer screening methods from women with or without the cervical disease. It also provides information about other sexually transmitted pathogens in women without cervical diseases.

HIV infection is the strongest predictor of HPV infection, whereby it greatly influences the high HPV prevalence and HPV viral load. We demonstrated a high HR-HPV burden of infection among women attending a rural community clinic, with 31% of the women being HIV-positive and 40.1% of those women having HR-HPV compared with 21.4% of the HIV-negative women. This is extremely high for a cohort where the median age was 46. The HPV infection was associated with age, the number of sexual partners and HIV infection. This data shows that HIV status and sexual behaviour influenced the prevalence of HR-HPV infection in this cohort. We further showed that HIV-positive women had a higher viral load of HR-HPV infection both with normal and abnormal cytology compared to HIV-negative women. The increase of HPV viral load among HIV-positive could be due to immunologic mechanisms. Furthermore, HPV viral load has been previously reported to increase with the severity of cervical cytology and can be used as the biomarker for cervical cancer progression (Moodley et al. 2009; Shen et al. 2014). These results show that education on risk factors associated with HPV infection is needed and highlights the importance of cervical cancer screening for both the general and high-risk populations.

In women with confirmed high-grade lesions (CIN2 & CIN3) on histology analysis had a high prevalence of HPV CIN2 (93.5%) and CIN3 (96.6%). There increase in HPV prevalence, and multiple HPV infection was influenced by the HIV status, with significantly higher rates observed among HIV-positive women than HIV-negative women. The top four identified HPV types were HPV35, HPV58, HPV45 and HPV16 for CIN2 women, while HPV35, HPV16, HPV33 and HPV58 were the most frequently detected genotypes for CIN3. HPV16 has been reported as the most dominant genotype in women with precancerous lesions and cervical cancer (Van Aardt et al. 2016), but this result was not observed in our study. It has been shown that cervical cancer lesions are associated with a single HPV type compared to multiple HPV types (van der Marel et al. 2015). In our study HPV35 was the predominant HPV type as a single HPV infection or in multiple HPV infections in both HIV-positive women and HIV-negative women. Multiple infections in this cohort might be due to the population studied (number of HIV-positive women) and the procedure used for collecting the specimens (exfoliative cervical specimens). The currently available vaccine does not cover HPV35. Therefore, this data shows that HPV vaccines will not be fully protective in this population and suggest the inclusion of this HR-HPV type in the next generation of HPV vaccines. HPV genotype data will raise awareness of HPV types present in women with precancerous lesions before implementing HPV testing strategies in this population.

Emerging evidence shows that the HPV test for self-sampling is an attractive, promising, and effective screening strategy for women residing in rural communities (Mbatha et al. 2017; Fitzpatrick et al. 2019; Esber et al. 2018). Data from this study shows that the detection of HR-HPV infection was comparable between self-collection and clinician-collection in both the rural community clinic and the referral clinic. The distribution of HPV types was similar, with HPV16, HPV35 and HPV33/52/58 being the most frequently detected genotypes in self-collected and clinician-collected specimens. HPV-specific type agreement between self-collection and clinician-collection varied from moderate to almost perfect. The compatibility of self-collection and clinician-collected for HPV detection indicate that the self-collection method can be incorporated and used as the alternative screening tool in the cervical cancer screening programme.

FTA cards as the specimen storage system provided good DNA quality for the detection of HPV infection, suggesting that self-collection on FTA cards is an effective storage method. HPV tests (HC-2 and *hpVIR* real-time PCR) used in this cohort had a good concordance for HPV detection, indicating that HPV testing can be included in cervical cancer screening for general and high-risk populations. However, implementation of HPV testing as the primary cervical cancer screening test would be challenging in our country due the high prevalence of HPV indicating that triage strategies would be useful. HC-2 offers cost-effectiveness but has the disadvantages of not having internal control for DNA and it does not specify which individual HR-HPV types are present in participants. The *hpVIR* real-time provides individual specific HPV types, HPV copy number, viral load, and control for DNA, but it might be complicated to implement this type of HPV test as it is an "in-house" test requiring a high level of laboratory expertise which is not available in most diagnostic centres in South Africa. Therefore, these findings encourage the need to conduct more studies comparing HPV tests in order to determine which test could be done on these samples.

The high level of acceptance of self-sampling among women from the community clinic and the referral clinic indicates that the self-collection method may positively impact this population. Implementation of self-sampling might increase the participation/uptake of cervical cancer screening in regions with limited equipment to perform cytology screening or women from rural areas who do not have access to screening. Self-sampling offers benefits to women. It gives women the opportunity to collect the specimen themselves without feeling embarrassed/uncomfortable, saves time and reduces the cost of traveling to the clinic/ hospital. However, women have raised some concerns about self-collection, such as fear of hurting themselves when collecting the samples, not collecting the samples correctly and the accuracy of the test. Other participants found self-sampling not appropriate for older and disabled women.

The interaction of HPV with other sexually transmitted pathogens influences the progression of cervical cancer lesions. *TV* was highly prevalent in this study, with low rates of the most

commonly detect STIs such as *CT* and *NG* in women from the community clinic, which could be influenced by the population studied. It is important to note that the population studied was not from the STI clinic. Pathobionts such as *UP* and *MH* were present at a higher rate, and high co-infection rates were found to be *UP*/*MH* (26%) and *UP*/HR-HPV (21%). Other studies have reported co-infection of these pathobionts and HPV infection (Camara et al. 2018; Parthenis et al. 2018). However there are no screening guidelines available for these pathogens. Furthermore, HIV infection and HSV-2 were associated with HR-HPV infection, which shows that this correctional may impact cervical cancer lesions' development. The high prevalence of STIs and other sexually transmitted pathogens shows that there is a need to improve STI screening, treatment, and implementing strategies to prevent and reduce the burden sexual transmitted pathogens.

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APPENDIX

Supplemental data

Supplemental Table 5.1: The association of sexually transmitted infections and behavioural factors.

Variables	STD prevalence % (n/N)	Univariate analysis OR (95%CI)	*P*-value
Age in years: median-45 years (IQR:38-53)			
HIV infection			
Negative	21.4% (27/126)	ref	ref
Positive	25.3% (20/79)	1.24 (0. 641-2.410)	0.520
Age category			
30-39 years	31.9% (22/69)	ref	ref
40-49 years	17.0% (10/59)	0.44 (0.187-1.018)	0.055
≥50 years	19.5% (15/77)	0.52 (0.242 -1.103)	0.088
Lifetime sexual partners			
1	9.7% (3/31)	ref	ref
2	19.7% (12/61)	2.29 (0.594-8.796)	0.229
≥3	28.3% (32/113)	3.69 (1.047-12.986)	**0.042**
Sexual partners in 12 months			
0	22.2% (12/54)	ref	Ref
≥1	23.2% (35/151)	1.06 (0.501-2.224)	0.886
Sexual partners in the last month			
0	24.7% (21/85)	ref	Ref
≥1	21.7% (26/120)	0.84 (0.437-1.626)	0.61
Used condoms during last sexual intercourse			
No	23.8% (30/126)	Ref	Ref
Yes	22.4% (17/76)	0. 92 (0.468-1.816)	0.814
Frequency of vaginal sex past 1 month			
0	23.8% (24/101)	ref	Ref
1-3	26.5% (18/68)	1.16 (0.569-2.343)	0.690
≥4	14.3% (5/35)	0. 54 (0.187-1.531)	0.243
Vaginal discharge (self-reported)			
No	20.0% (19/95)	ref	ref
Yes	25.5% (28/110)	1.37 (0. 705-2.645)	0.355
Frequency of vaginal discharge			
Current/last week	18.9% (7/37)	ref	ref
More than a week and less than 6 months	26.1% (6/23)	1.52 (0. 437-5.238)	0.514
More than or equal to 6 months	28.6% (14/49	1.71 (0. 612-4.802)	0.305
Using any contraception with current partner			
No	19.2% (23/120)	ref	ref
Yes	29.3% (24/82)	1.75 (0. 904-3.370)	0.097
HR-HPV infection			
Negative	20.9% (29/139)	ref	ref
Positive	27.3% (18/66)	1.42 (0. 721-2.804)	0.309

HR-HPV: high-risk human papillomavirus, **OR**: odds ratio, **CI**: confidence intervals, **ref**: reference**, Highlighted values**: significant p-value, **ref**: reference; **n**: number of women responded, **N**: total number of study participants.

STIs being analysed for this table: *Chlamydia trachomatis*, *Herpes simplex virus-2*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis*.

Supplemental Table 5.2: The association of human papillomavirus infection and behavioural factors.

	HR-HPV prevalence	Univariate analysis		Multivariate analysis	
Variables	% (n/N)	**OR (95%CI)**	***p*-value**	**OR (95%CI)**	***p*-value**
Age in years: median-45 years (IQR:38-53)					
HIV infection					
Negative	25.4% (32/126)	ref	ref	ref	ref
Positive	43.0% (34/79)	2.22 (1.219-4.042)	**0.009**	1.95 (0.790-4.827)	0.147
Age category					
30-39 years	40.6% (28/69)	ref	ref	ref	ref
40-49 years	37.3% (22/59)	0.87 (0.426-1.777)	0.704	1.28 (0.469-3.522)	0.627
≥50 years	20.8% (16/77)	0.38 (0.185-0.797)	**0.010**	0.81 (.266-2.482)	0.717
Lifetime sexual partners					
1	16.1% (5/31)	ref	ref	ref	ref
2	31.2% (19/61)	2.35 (0.783-7.065)	0.127	0.94 (0.150-5.932)	0.951
≥3	37.2% (42/113)	3.08 (1.098-8.619)	**0.033**	1.42 (.235- 8.583)	0.702
Sexual partners in 12 months					
0	24.1% (13/54)	ref	ref		
≥1	35.1% (53/151)	1.71 (0.841-3.461)	0.139		
Sexual partners in the last month					
0	28.2% (24/85)	ref	ref		
≥1	35.0% (42/120)	1.37 (0.749-2.502)	0.308		
Used condoms during last sexual intercourse					
No	31.0% (39/126)	ref	ref		
Yes	34.2% (26/76)	1.16 (0.633-2.126)	0.631		
Frequency of vaginal sex past 1 month					
0	30.7% (31/101)	ref	ref		
1-3	29.4% (20/68)	0.94 (0.481-1.841)	0.859		
≥4	40.0% (14/35)	1.50 (0.678-3.342)	0.315		
Vaginal discharge (self-reported)					
No	31.6% (30/95)	ref	ref		
Yes	32.7% (36/110)	1.05 (0.586-1.898)	0.861		
Frequency of vaginal discharge					
Current/last week	43.2% (16/37)	ref	ref		ref
More than a week and less than 6 months	34.8% (8/23)	0.70 (0.239-2.055)	0.516	0.76 (0.246-2.316)	0.624
More than or equal to 6 months	22.5% (11/49)	0.38 (0.149-0.967)	**0.042**	0.38 (0.141-1.029)	0.057
Using any contraception with current partner					
No	30.8% (37/120)	ref	ref		
Yes	35.4% (29/82)	1.23 (0.676-2.227)	0.500		

HR-HPV: high-risk human papillomavirus, **OR**: odds ratio, **CI**: confidence intervals, **ref**: reference**, Highlighted values**: significant p-value, **ref**: reference; **n**: number of women responded, **N**: total number of study participants.

Form for questionnaire

Name of facility: …….

Date: ……

Study number: *Place sticker*

1. Date of Birth _____DDMMYYYY ☐☐/☐☐/☐☐☐☐

2. How old are you (Years)? _____☐☐years

I would like to ask you details about your education and employment.

3. What is the highest level of education that you have completed? _____☐☐

0. Did not attend school at all	7 Std 5 Grade 7
1. Sub A Grade 1	8 Std 6 Grade 8
2. Sub B Grade 2	9 Std 7 Grade 9
3. Std 1 Grade 3	10 Std 8 Grade 10
4. Std 2 Grade 4	11 Std 9 Grade 11
5 Std 3 Grade 5	12 Std 10 Grade 12
6 Std 4 Grade 6	

4. Did you have any training of a year or more after school?

A. Yes
B. No

IF YES,

4a. How many years at

i. University_____☐☐years

ii. Technical College/Technikon _____☐☐years

iii. Other, Specify _____☐☐years

5. Do you do work that you are paid for?

A. Yes
B. No

IF YES,

5a. What work do you do?

Specify _____

IF NO,

5b. Are you ☐

1. Unemployed-looking for work

2. Unemployed-not looking for work
3. Home-maker (by choice)
4. Full-time student
5. Disabled (physically or mentally) or a pensioner (government or private civil pension/not working due to old age)

6. What is the approximate total household income per month?

(This money could be coming from grants and donations from various sources) **R**

The next questions ask about tobacco use.

7. 1. Do you now or have you ever smoked cigarettes?

i. Never_____ ☐

ii. Ex-smoker _____ ☐

iii. Present smoker _____ ☐

8. Have you ever tried cigarette smoking, even one or two puffs?
   A. Yes
   B. No

9. How old were you when you smoked a whole cigarette for the first time?____ ☐☐ years

10. During the past 30 days, on how many days did you smoke cigarettes?
   A. 0 days
   B. 1 or 2 days
   C. 3 to 5 days
   D. 6 to 9 days
   E. 10 to 19 days
   F. 20 to 29 days
   G. All 30 days

11. During the past 30 days, on the days you smoked, how many cigarettes did you smoke **per day**?
   A. I did not smoke cigarettes during the past 30 days
   B. Less than 1 cigarette per day
   C. 1 cigarette per day
   D. 2 to 5 cigarettes per day
   E. 6 to 10 cigarettes per day
   F. 11 to 20 cigarettes per day
   G. More than 20 cigarettes per day

The next questions ask about drinking alcohol. This includes drinking beer, wine, wine coolers, liquor, brandy, and traditional beer.

12. Did you ever try to drink alcohol?
    A. Yes
    B. No

13. How old were you when you had your first drink of alcohol?
    A. I have never had a drink of alcohol other than a few sips
    B. 8 years old or younger
    C. 9 or 10 years old
    D. 11 or 12 years old
    E. 13 or 14 years old
    F. 15 or 16 years old
    G. 17 years old or older

14. During the past 30 days, on how many days did you have at least one drink of alcohol?
    A. 0 days
    B. 1 or 2 days
    C. 3 to 5 days
    D. 6 to 9 days
    E. 10 to 19 days
    F. 20 to 29 days
    G. All 30 days

15. During the past 30 days, on how many days did you have 5 or more drinks of alcohol in a row, that is, within a couple of hours?
    A. 0 days
    B. 1 day
    C. 2 days
    D. 3 to 5 days
    E. 6 to 9 days
    F. 10 to 19 days
    G. 20 or more days

The next questions ask about sexual behaviour.

16. Have you ever had sexual intercourse?
    A. Yes
    B. No

17. How old were you when you had sexual intercourse for the first time? ____☐☐years

18. What is the age difference between you and your current sexual partner(s)
    A. …. years older
    B. …. years younger

19. What was the age difference between you and your past sexual partner(s)
    A. …. years older
    B. …. years younger

20. During the past year, with how many people have you had sexual intercourse? __☐☐

21. During the past 1 month, with how many people did you have sexual intercourse?☐☐

22. During your life, with how many people have you had sexual intercourse? _____☐☐

23. How many NEW sexual partners have you had in the past 1-year?_____☐☐

24. Did you drink alcohol or use drugs before you had sexual intercourse the **last time**?
    A. I have never had sexual intercourse
    B. Yes
    C. No
25. The **last time** you had sexual intercourse, did you or your partner use a condom?
    A. I have never had sexual intercourse
    B. Yes
    C. No
26. The **last time** you had sexual intercourse, what **one** method did you or your partner use to **prevent pregnancy**? (Select only **one** response.)
    A. I have never had sexual intercourse
    B. No method was used to prevent pregnancy
    C. Birth control pills
    D. Condoms
    E. An intrauterine device (such as Mirena or ParaGard) or implant (such as Implanon or Nexplanon)
    F. 3-month injectable (‘depo’)
    G. 2-month injectable (‘nuristerate’)
    H. Withdrawal or some other method
    I. Injectable but don’t know which one
27. How many times have you had VAGINAL sex in the past 1-month? _____
28. How many times have you had ANAL sex in the past 1-month? _____
29. How many times have you had ORAL sex in the past 1-month? _____
30. Are you or your partner currently using any kind of contraception?
    A. Yes
    B. No
    C. Don’t know
31. Which of the following methods of contraception are you (or your partner) <u>using currently</u>?

    A. I have never had sexual intercourse
    B. No method was used to prevent pregnancy
    C. Birth control pills
    D. Condoms

E. An intrauterine device (such as Mirena or ParaGard) or implant (such as Implanon or Nexplanon)
F. 3-month injectable ('depo')
G. 2-month injectable ('nuristerate')
H. Withdrawal or some other method
I. Injectable but don't know which one

32. How old were you when you started taking contraceptives_____ ☐☐years

33. Have you ever been pregnant?

A. Yes
B. No

IF YES, How many live children do you have? _____

34. Have you had any miscarriages, abortions/ectopic pregnancy?

A. Yes
B. No

IF YES, how many _____

The next 2 questions ask about your genital health

35. Have you had vaginal discharge that causes itching or foul smell that has caused you some worry?

A. Yes
B. No

IF YES, When was the last time it occurred?

A. in the last week
B. more than 1 week but less than a month ago
C. more than 1 month but less than 6 months
D. more than 6 months ago

36. Have you had ulcers/blisters/warts on the genitals

A. Yes
B. No

IF YES, When was the last time it occurred?

E. in the last week
F. more than 1 week but less than a month ago
G. more than 1 month but less than 6 months
H. more than 6 months ago

I would like to ask you about your Pap smear

Prior to today had you heard of a Pap smear? Can you describe this for me?
If the person fully understands don't give an explanation again.

Otherwise say, "Let me go over it again" and then give explanation*:*

It is a test to detect abnormal cells in the mouth of the womb that could lead to cancer.

When performing this test the doctor or nurse places an instrument called a speculum (spoon) in the woman's vagina so that he/she can see the mouth of the womb and the test is done.

Then interviewer to ask:

37. Did you ever have this test done?

A. Yes
B. No
C. Unknown

IF YES:

37a. How many times have you ever had a PAP? _____ ☐☐ years

37b. Age at first? _____ ☐☐ years

37c. Age at last? _____ ☐☐ years

37d. Did you ever get the result of any of your PAP smears/tests?
A. Yes
B. No

IF YES:

37e. What were you told: _____

37f. Have you ever been told that there was something wrong with the mouth of your womb?
A. Yes
B. No

IF YES:

37g. What were you told:_____

Nurse's name (please print) ..... Nurse's signature ..... Date .....

Nurse's comments:

<u>Appendix 5 - Pelvic examination, list of specimens collected and HIV rapid test results</u>

1. Pelvic Examination

Symptoms	**Present/**	**Absent**	**Comment**	**Code**
Warts				
Discharge				
Other observations				

2. Specimens collected - tick when completed

Self-collected cervical-vaginal specimen on FTA card	
Pap smear specimen	
Clinician collected cervical specimen on FTA card	
Clinician collected cervical specimen in STM	
Anal specimen	
Oral specimen	

3. Rapid HIV test results

Offer HIV pre-test counselling

HIV Rapid Test Result _____Positive ☐ Negative ☐

Offer HIV Post-test counselling

To be completed if participant is HIV positive and was aware of HIV status

Are you taking anti-retrovirals (ARVs)

A. Yes
B. No

3a. What are the names of the ARVS that you are taking _____?

(Nurse also check the clinic records for this information even if the participant gave the name of ARVs)

If participant is HIV positive and was the first time to know her HIV status please refers to the Community Health Centre for further management.

Nurse's name (please print) ..... Nurse's signature ..... Date .....

Nurse's comments:

Appendix 6 - Self-sample and clinician-collected specimen; and exit form

Date of Visit: __ __ / __ __ / __ __ __ __ Name of Interviewer: _____

DD MM YYYY

1. How did you feel when collecting the first sample yourself (self-sample)?

	Extremely	Very	Moderately	Slightly	Not at all
a) Embarrassed	1	2	3	4	5
b) Self-confident	1	2	3	4	5
c) Discomfort	1	2	3	4	5
d) Ignored by health worker	1	2	3	4	5
e) Intrigued/interested	1	2	3	4	5

2. How did you feel when the doctor was collecting the other samples?

	Extremely	Very	Moderately	Slightly	Not at all

a)	Embarrassed	1	2	3	4	5
b)	Self-confident	1	2	3	4	5
c)	Discomfort	1	2	3	4	5
d)	Ignored by health worker	1	2	3	4	5
e)	Intrigued/interested	1	2	3	4	5

3. **Would you be willing to collect a sample yourself (self-sample) at home and bring it in for testing?**
    a. Yes
    b. Not sure
    c. No
    d. If no, why not? _____

4. **Which method do you prefer for cervical cancer screening?**
    a. To take a sample myself
    b. For a health worker to take a sample
    c. Either for myself to take a sample or a health worker to take a sample
    d. Neither

5. **What comments do you have on your experience in this study today?**

_____
_____
_____
_____