HUMAN PAPILLOMAVIRUS (HPV) INFECTION AND ITS ASSOCIATION WITH CERVICAL DYSPLASIA AMONG HIV POSITIVE WOMEN ATTENDING ART CLINIC, KORLE-BU TEACHING HOSPITAL, ACCRA

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DECLARATION

I hereby declare that this submission is my own original and novel work towards a Doctor of Philosophy Degree (PhD.) and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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ABSTRACT

Globally, about 35 million people are living with HIV of which 16 million are women aged 15 years and above, 80% of whom live in the Sub-Saharan African region These women living with HIV (WLHIV), especially those having low CD4 T-cell counts are more likely to be infected with human papillomaviruses (HPV), which are the major cause of cervical cancer and it's precursor lesions. But less is known about the prevalence of HPV infection and the specific types involved in this particular population of women in Ghana.

A cross-sectional study was carried out to establish the distribution of genital HPV genotypes among an unscreened population of WLHIV attending a special HIV outpatient clinic at the Korle-Bu Teaching hospital (KBTH) Accra, Ghana. Cervical swabs were available for 538 eligible women.

A nested multiplex PCR (NMPCR) assay was conducted for the detection and typing of HPV genotypes 6/11, 16,18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66, and 68. Cervical smears were also prepared and examined independently for cytologic abnormalities by two cytologists and confirmed by a pathologist.

HPV DNA was detected in 52.0% of all cervical swabs tested, the prevalence of LR (Low Risk) HPV was 10.2% (95% CI: 11.3 – 17.6). while that of HR (High Risk) HPV was 29.7% (95% CI: 27.4 – 35.7). The commonest HR types were, HPV-35 (14.1%), HPV-18 (13.0%;), HPV-58 (7.4%;), HPV-52 (7.2%;), HPV-45 (4.8%;) and HPV 42, a low-risk type, was also common (7.8%;). The frequency of HPV 16 was 2.4%. Cytologicaly, 2.8% had ASCUS, 3.9% LGSIL and 2.8% HGSIL, None of the women was found to have atypical glandular cells or adenocarcinoma.

There is a high burden of HPV in women living with HIV attending clinic at the KBTH.

I recommend that screening for cervical dysplasia should be done for WLHIV routinely as part of their comprehensive care.

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ABBREVIATIONS

ACIP	Advisory committee on immunization practices
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
ART	Antiretroviral Therapy
ASC	Atypical Squamous Cells
ASC-H	Atypical Squamous Cells, cannot rule out HSIL
ASC-H	Atypical squamous cells, cannot rule out HSIL
ASCUS	Atypical Squamous cells of Undetermined Significance
ASCUS	Atypical squamous cells of undetermined significance
cART	Combination antiretroviral therapy
CC	Cervical Cancer
CIN	Cervical squamous Intraepithelial Neoplasia
dATP	Deoxyadenosine Triphosphate
dCTP	Deoxycytidine Triphosphate
dGTP	Deoxyguanosine Triphosphate
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide Triphosphate
ECA	Epithelial Cell Abnormality
HAART	Highly Active Antiretroviral Therapy
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HR HPV	High risk human papillomavirus
HSIL	High-grade Squamous Intraepithelial Lesion
IARC	International Agency of Research on Cancer
ICC	Invasive Cervical Cancer
KBTH	Korle-Bu Teaching Hospital
LCR	Long Control Region
LR HPV	Low risk human papillomavirus

LSIL	Low-grade Squamous Intraepithelial Lesion
NCR	Noncoding Region
ORF	Open Reading Frame
SSA	Sub-Saharan African
STI	Sexually transmitted infection
URR	Upstream Regulatory Region
VIA	Visual inspection with acetic acid
VILI	Visual inspection with lugols iodine
WLHIV	Women living with HIV
YLL	Years of life lost

Chapter 1
INTRODUCTION

1.1 BACKGROUND

Globally, about 35 million people are living with HIV of which 16 million are women aged 15 years and above, 80% of whom live in the Sub-Saharan African region (HIV). Low- and middle-resource countries, where women have been hit hardest by the Acquired immune deficiency syndrome (AIDS) epidemic, have historically also had a very high prevalence of human papillomavirus (HPV) infection (Franceschi and Jaffe, 2007). HIV cases in Africa is still on the rise among women due to cultural and heterosexual activities (Awofala and Ogundele, 2018). In 2015, approximately 390,000 new infections of HIV were recorded among young women between the ages of 15 to 24 representing about 60% of all new HIV infection all over the world in this age group (HIV/AIDS, 2018) A number of studies have established that, women living with HIV, especially those having low CD4 T-cell counts are more likely to be infected with human papillomaviruses (HPV) (Burd, 2003b; Strickler *et al.*, 2005), which are the major cause of cervical cancer and it's precursor lesions (Tornesello *et al.*, 2014) Women living with HIV also have a higher rate of infection with high-risk HPV types, multiple HPV infections and there is also the tendency for the infection to persist for a longer period thus, the likelihood of developing cancer of the cervix (Burd, 2003b; Strickler *et al.*, 2005; Clifford *et al.*, 2006d; Odida *et al.*, 2011; Akarolo-Anthony *et al.*, 2013). It is also well known that, HIV infection itself increases the risk of developing cervical squamous intraepithelial neoplasia (CIN) and cancer of the cervix (Franceschi and Jaffe, 2007)

Human papillomaviruses (HPVs) are DNA viruses that infect epithelial (skin and mucosal) cells and they are the most common sexually transmitted infection in both males and females worldwide. They are a heterogeneous group of viruses with circular double-stranded DNA genomes about 8,000 nucleotides in size (SCHEURER *et al.*, 2005) All human papillomavirus

genomes include 3 general regions: (1) an upstream regulatory region (URR), which contains sequences that control viral transcription and replication; (2) an early region, which contains open reading frames (ORFs; e.g. E1, E2, E4, E5, E6 and E7) involved in multiple functions including trans-activation of transcription, transformation, replication, and viral adaptation to different cellular milieus, and (3) a late region, which codes for the L1 and L2 capsid proteins which form the structure of the virion and facilitate viral DNA packaging and maturation. All HPVs described to date contain an E1, E2, E4, L1, L2 and some E6/E7-like functions (Burk *et al.*, 2009).

HPV is a major cause of sexually transmitted diseases in both sexes worldwide, with varying degree of prevalence among groups and regions of habitation (De Sanjosé *et al.*, 2007). Infections are mostly asymptomatic and are normally found among sexually active individuals at a point in their lives (Trottier and Franco, 2006; Luchters *et al.*, 2010). Women are at risk and are commonly infected by multiple strains of the virus at young age which makes HPV infection more prevalent among such group as they become sexually active. The prevalence then reduces gradually as they increase in age which may likely be due to the building up of immunity against the virus and reduction in sexual intercourse among them (Khan *et al.*, 2005). Human papillomavirus have two main groups: those found on the surface of the skin and those found in the anogenital tract and the mucosa of the mouth, throat and respiratory tract (Burd, 2003b). Some HPVs causes genital warts, while others are responsible for premalignant squamous intraepithelial lesions of different grades and bad lesions, which are major risk factor for invasive cervical cancers (Burd, 2003b) However, only a few HPV-infected individuals progress to invasive cervical cancer. Most infected individuals eliminate the virus without developing recognized clinical manifestation.

Infection with HPV is also an indication of the risk of cervical and anal cancer in females and anal cancer in homosexual males (Chaturvedi, 2010; Doorbar *et al.*, 2012). Infection in the cervix is found in the transformation zone. The virus must reach the basal cell layer for infection to be initiated, this process normally takes approximately 24 hours to occur.(Crosbie *et al.*, 2013)

In the year 2000, sub-Saharan Africa experienced an estimated 57, 000 cases of cervical cancer (22% of cases of cancer among women) and an age-standardized incidence of 31cases per 100,000 women (Zeier *et al.*, 2012). Furthermore, the cervical cancer incidence has not decreased in any region of sub-Saharan Africa in recent decades; in fact, significant upward trends have been reported in several areas (Yakasai *et al.*, 2012; Kayumba, 2014; Wanyoike-Gichuhi *et al.*, 2014; Donkoh *et al.*, 2019b). Because both HPV and HIV infection are sexually transmitted, the two infections are often found together (Clifford *et al.*, 2006b; Park *et al.*, 2014).

In addition, as a result of HIV-induced immune impairment, there is also an increased probability that HPV infection will become persistent in HIV-infected women (Park *et al.*, 2014) and evolve into precancerous and cancerous lesions of the cervix uteri (Mbulaiteye *et al.*, 2006). As expected, the relative risk of invasive cervical cancer among women living with HIV infection varies from country to country on the basis of the extent to which premature death due to other causes or early detection of cancer prevents progression of pre invasive lesions to the invasive stage (Franceschi and Jaffe, 2007; Sloan and Gelband, 2007).

Effective screening and early treatment of precancerous cervical lesions are, thus, key factors in preventing cervical cancer in HIV-infected women (Humans *et al.*, 2007). High-resource countries have been able to prevent cervical cancer among women with HIV/AIDS to different

extents. In the United States, for example, a high percentage of women (about 81%) who receive treatment for HIV undergo annual Papanicolaou smears (Mbulaiteye *et al.*, 2011) and the vast majority (94%) of cases of cervical carcinoma among these women are detected at the in-situ stage (Frisch *et al.*, 2000).

1.2 PROBLEM STATEMENT

Cytological screening of the cervix using the Pap smear test and the early detection of HPV play an important role in the secondary prevention of ICC, thereby reducing HPV-associated mortality (WHO/ICO, 2010b). However, due to a lack of effective screening programs in low- and middle-income countries, including Ghana, cervical cancer precursor lesions most often go undetected resulting in development of ICC with its attendant high mortality rates, in these settings.

As both HIV infection and HPV infection are sexually transmitted, the two infections are often found together in populations with a high risk for sexually transmitted infections (Sahasrabuddhe *et al.*, 2007; Bernard *et al.*, 2010). Although most women who develop natural HPV infections have a capacity to clear the infections in a few months, HIV-infected women remain at a continued substantial risk for cervical cancer, even if they receive ART because these treatment programs improve their survival long enough for cervical cancer precursors to manifest and progress to invasive cancer (Mbulaiteye *et al.*, 2006; Sinayobye *et al.*, 2014). With the introduction of highly active ART (HAART), women living with HIV are expected to live longer – an infected 25 year-old-woman has an estimated average survival age of 73.9 years therefore if this woman lives that long, HPV persistence may lead to her developing

cervical abnormalities resulting in cervical cancer, to prevent this, other strategies like regular cervical screenings are urgently needed (Ghebre *et al.*, 2017).

Ghana does not have a national cervical cancer screening program presently and therefore Pap smears are routinely ordered for differential diagnosis in cases of unusual vaginal bleeding rather than as part of a routine gynaecologic investigation or screening program (Adanu, 2002). As such, data on cervical intraepithelial neoplasia and invasive cancer among women living with HIV and enrolled/unenrolled on HAART is routinely not available.

Unfortunately, knowledge on the epidemiology of HPV virus among women living with HIV in Ghana is limited. It is also well known that there are regional differences in prevailing HPV genotypes, (Clifford *et al.*, 2003; de Sanjose *et al.*, 2010; Forman *et al.*, 2012; Pirog *et al.*, 2014) therefore, there is the need to know the specific prevailing HPV genotypes among women living with HIV in Ghana. No policy on the prevention of cervical cancer has been established among women living with HIV therefore it is very important that the local differences and distribution of HPV genotypes among this group of women be known to help inform policy makers for implementing monitoring and evaluating a cervical cancer prevention program in Ghana.

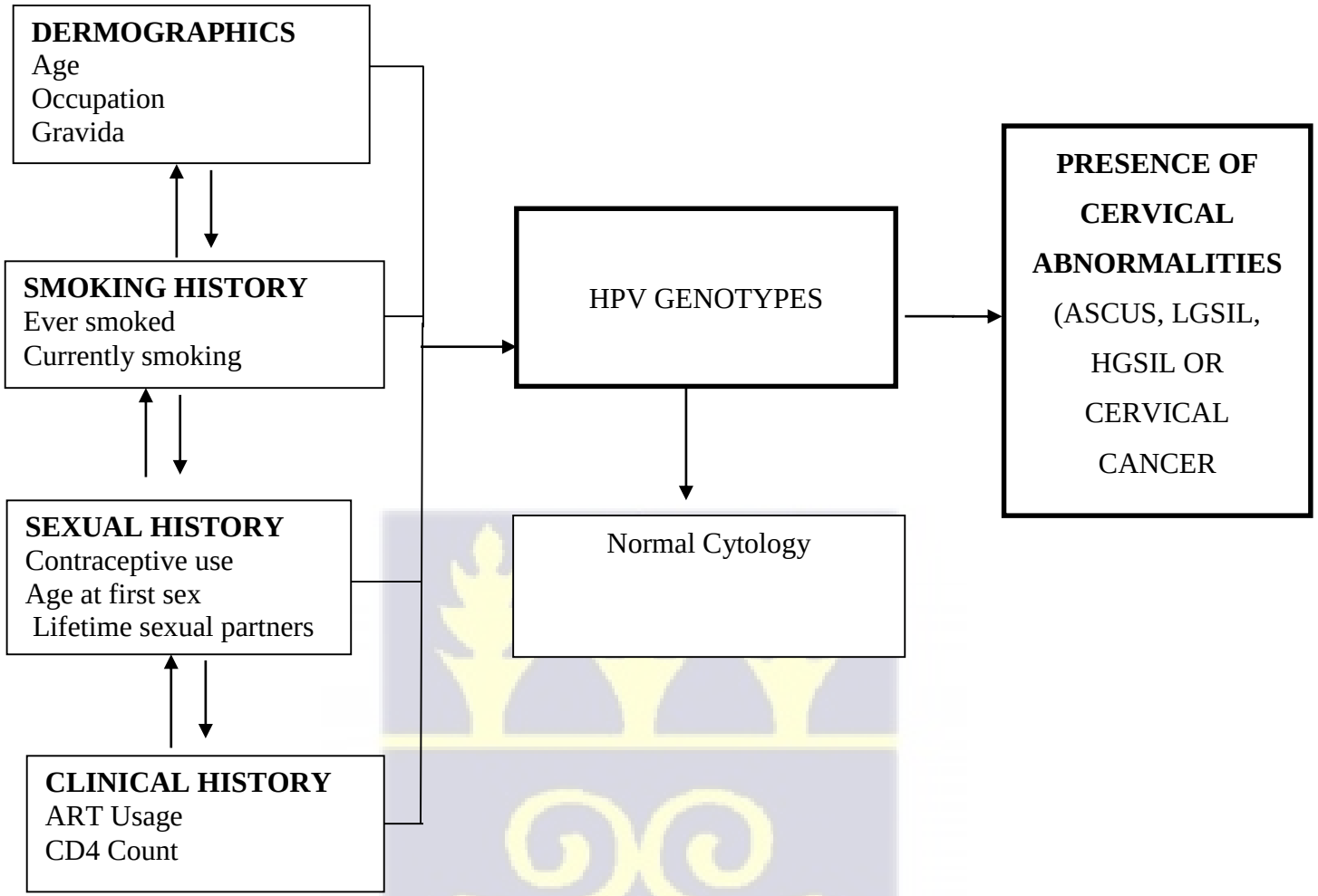

Figure 1.2-1: Conceptual framework of the interactions of socio-demographic parameters, smoking history, sexual history, clinical history and HPV infection on the pathogenesis of cervical cancer and its precursor lesions among women living with HIV.

1.3 CONCEPTUAL FRAMEWORK

Figure 1.2.1 looks at the factors associated with acquiring HPV infection and the relationship to progression to precancerous lesions and cervical cancer in women living with HIV. Infection with HPV is a necessary and often sufficient cause of cervical cancer. However, not all HPV infections lead to observable cervical lesions as most viral infections clear naturally over the course of time usually spanning 6-18 months (Castle *et al.*, 2009). In most cases (90% of) infections clear spontaneously within 24 months (Schiffman *et al.*, 2007). Once an infection is cleared, it is undetectable by cytological assessment. Evidence from natural history studies reveal that persistent infection with human papillomavirus (HPV) is a necessity for the development of ICC and precursor lesions (Kjaer *et al.*, 1996; Walboomers *et al.*, 1999). Apart from persistent HPV infection, other genetic, viral and population factors have been suspected to play a role in the development of cervical cancer and the development of precancerous lesions (Schlecht *et al.*, 2001; Trottier *et al.*, 2006b; Bosch *et al.*, 2008; Wang *et al.*, 2010). Non-viral factors like a woman's age, menstrual status and immunosuppression are also likely to influence the outcome of an HPV infection (Bosch *et al.*, 2006). Cytological screening of the cervix using the Pap smear test and the early detection of HPV in cross-sectional studies can reveal HPV genotypes associated with the spectrum of cytological disease.

1.4 JUSTIFICATION

The attribution of individual HPV types to cervical intraepithelial lesions varies ethno-geographically (Vaccarella *et al.*, 2006). Since the genotype profile of HPV infection differ from high-risk population to another, the epidemiology of human papillomaviruses have been found to differ from one region to another as well. Among populations of HIV infected women and

commercial sex workers, diverse HPV genotypes may be prevalent depending on several potential confounders. In a previous study evaluating the concordance of HPV infection among heterosexually active couples and the impact of HIV coinfection on the prevalence of HPV, HIV coinfection in one partner had a significant impact on the prevalence of HPV infection in the other partner (SCHEURER *et al.*, 2005). Concordance of the same HPV genotypes was more commonly found among couples where one or both partners were HIV-infected, compared with HIV-uninfected couples (SCHEURER *et al.*, 2005). The majority of HIV-infected women were infected by their male sexual partners in Korea (SCHEURER *et al.*, 2005).

As such, HPV genotyping studies among HIV cohorts allow us to establish and compare HPV genotype distributions in populations with different risk factor profiles, to investigate associations with cervical pre-cancerous and (to a lesser extent cancerous lesions) and to appreciate the behaviour of individual HPV genotypes in this population.

Furthermore, HPV typing is of importance for characterizing study populations and for monitoring the efficiency of HPV-targeted therapies and vaccines in high-risk populations (van den Brule *et al.*, 2002). Issues related to the epidemiology of HPV infection in this population of women will provide the basis for evaluating the potential risk of spillover of HPV genotypes into the general population and policy decisions regarding HPV vaccination as a national strategy for cervical cancer prevention, and to determine the specific socio-demographic groups that would need to be aggressively targeted for vaccination to obtain optimum protection. Reliable knowledge of the distribution of specific HPV types along the spectrum of cervical abnormalities will allow us to estimate the burden due to HPV types that are vaccine preventable after taking into account the extended cross-protection offered by available vaccines, and as a

matter of fact, the effect of vaccination in Ghana. It has been strongly inferred that HPV testing would be indispensable in an era of HPV vaccination (Franco *et al.*, 2006).

HIV-infected women in various settings were found to have higher rates of persistent HPV infection, more abnormal Pap tests, more cervical intraepithelial neoplasia (CIN) lesions, and aggressive cervical cancer, compared with the general population, and these numbers are increasing (Lörincz and Richart, 2003; Burk *et al.*, 2009). Up to 20 % of HIV coinfected patients develop HPV-induced premalignant lesions of the uterine cervix within three years of HIV diagnosis (Jamieson *et al.*, 2002). Progression of an untreated HPV-induced dysplastic lesion can lead to invasive cervical cancer, an AIDS defining illness (Chaturvedi *et al.*, 2009) The high rate of cervical disease reported in HIV-infected women and the extended life expectancy due to better access to antiretroviral therapy (ART) have led to recommendations for aggressive screening and prompt treatment of cervical lesions; yet, the most appropriate strategy to screen HIV infected women, particularly in resource-constrained settings, remains unclear (Cuzick *et al.*, 2003; Zielinski *et al.*, 2004; Clifford *et al.*, 2006b). Moreover, the existing evidence on the impact of ART on the incidence and evolution of CIN is still unclear, thus reinforcing the recommendation of systematic screening of all HIV-infected women, even those on ART (Burk *et al.*, 2009; Unaids, 2010). Overall, the evidence assessing the extent and magnitude of HPV infection and the burden of cervical abnormalities and cancer among HIV-infected women is sparse and remains largely undocumented. The paucity of literature in this population especially in the HAART era motivated this study.

Thus the need for data on HPV prevalence and genotype distribution in Ghana based on sensitive and specific molecular biomarkers of infection, coupled with the need to investigate the effects of ethno-geographic and demographic variables on the prevention of cervical cancer in Ghana, particularly the impact of vaccination, motivated the present work. It is the first study to report the type-specific distribution of HPV in cervical disease in the Greater Accra region of Ghana, using a reliable and highly practicable molecular assay. It is envisaged that the outcome of this novel study would provide evidence-base information and incidence data that will impact future vaccination programme in Ghana and perhaps influence cervical prevention and treatment.

1.5 RESEARCH QUESTIONS

1. What is the proportion of women living with HIV with cervical abnormalities and cervical cancer?
2. What is the proportion of women living with HIV with HPV infection? Which HPV genotypes are commonly found among women living with HIV in Ghana?
3. What is the relationship between HPV genotypes and the presence of cervical abnormalities and cancer if any among women living with HIV?
4. What are the risk factors for abnormal pap smears among women living with HIV?
5. What are the risk factors for HPV positivity among women living with HIV?

1.6 GENERAL OBJECTIVE

This study aims to determine the prevalence and distribution of HPV genotypes and associated cervical disease in an immunodeficiency care setting in Accra, in the Greater Accra Region of Ghana.

1.6.1 SPECIFIC OBJECTIVES

Specific Objective 1: To determine the proportion of HIV positive women with cervical cancer and precursor cell abnormalities at the Korle-Bu Teaching Hospital, Accra.

Specific Objective 2: To assess the proportion of HIV positive women with HPV infection at the Korle-Bu Teaching Hospital, Accra**.**

Specific Objective 3: To determine the association between high-risk HPV genotypes and abnormal cytology among HIV positive women attending clinic at the Korle-Bu Teaching Hospital, Accra.

Specific Objective 4: To identify the factors associated with abnormal cytology among HIV positive women attending clinic at the Korle-Bu Teaching Hospital.

Specific Objective 5: To identify the factors associated with high-risk HPV genotype infection among HIV positive women attending clinic at the Korle-Bu Teaching Hospital.

Chapter 2

LITERATURE REVIEW

2.1 HISTORY OF PAPILLOMAVIRUSES (PVs) IN CANCER

As early as 1842, an Italian physician became curious of the increase in mortality as a result of cancer of the cervix that were being recorded in married women, widows and prostitutes living in Verona during the period 1760-1839 after he reviewed their death certificates (Gasparini and Panatto, 2009). Death as a result of cervical cancers was not common in virgins and nuns. This led him to conclude that, whatever was responsible for the cervical cancer that were killing these married women, widows and prostitutes and not virgins and nuns was sexually transmitted. Studies on papillomavirus started in the early twenties when in 1933 it was isolated as the possible causal agent of warts in rabbits. Since then, papillomaviruses have been accepted as a natural infectious agents for the development of warts in various mammals including man (Franceschi and Jaffe, 2007). The warts in rabbits were described as having the potential for malignant transformation by Rous in 1935 and the first visualization of human papillomavirus by electron microscopy was reported in 1949 by Strauss *et al*. In 1950, the carcinogenic potential of HP virus was established.

The viral genomic structure of HPV was first demonstrated in 1963 (Crawford & Follett, 1967). Harald cited that *Herpes simplex* virus type 2 was initially the causative agent for cervical cancer (Awofala and Ogundele, 2018). It was not until the early 1970s that studies on the possible role of HPV's in cancer was started. Determination of HPV causation of cervical cancer started in 1972 when it was reported in anecdotal reports in the medical literature of rare malignant conversion of genital warts (*Condylomata acuminata*) into squamous cell carcinomas (zur Hausen, 1977). It was observed that, as many as 70% of

cervical dysplasia might be associated with the so-called koilocytotic atypia which was a hallmark of HPV infection (Basnet *et al.*, 2018). In November 1991, a workshop convened by the International Agency for Research on Cancer (IARC) and the World Health Organization (WHO) officially concluded that, based on the epidemiological and laboratory data, the association between HPV and cervical cancer was beyond reasonable doubt, and infection with the virus should be considered as cause to the development of cervical cancer (Bosch *et al.*, 1992)

2.2 DIVERSITY OF PAPILLOMAVIRUSES AND EPITHELIAL TROPISMS

More than 200 papillomaviruses have been identified and have been completely sequenced based on DNA sequence homology instead of conventional serology (de Villiers, 2013). Out of this, approximately 150 are human papillomaviruses (HPV) (Bernard *et al.*, 2010). In recent years, sensitive detection methods have permitted the identification of a large number of new HPV types (primarily Beta and Gamma types) from swabs taken from cutaneous epithelium (de Villiers, 2013). By convention, but without official seal, genotypes are considered distinct if they share less than 90% homology in the DNA sequence of the open reading frame (ORF) coding for the major (L1) capsid protein (Manhart and Koutsky, 2002; Yu *et al.*, 2022). The traditional papillomavirus types a species share between 71 and 89% nucleotide sequence identity within the complete L1 ORF and subtypes have between 90% and 95% homology, and variants between 96% and 98% (De Villiers *et al.*, 2004; Brancaccio, 2020).

The taxonomy of papillomaviruses, which was initially based on genomic cross-hybridizations and restriction patterns is now a system based on phylogenetic algorithms of sequence homology because papillomavirus genomes are thought to be very static, and sequence mutations or recombination are very rare events: mutational changes are thought to occur at frequencies similar to those of the DNA genomes of the infected host organism (Cogliano *et al.*, 2005; McBride, 2021). Phylogenetically, papillomaviruses are recognized as belonging to the family Papillomaviridae having 18 genera(Fang *et al.*, 2020). The approximately 40 types able to infect the human ano-genital tract (De Villiers *et al.*, 2004; Garutti *et al.*, 2018), collectively called the genital HPVs, belong to the alpha genus. Clusters of lower order are known as species; they are closely related phylogenetically and have similar biological properties but not necessarily oncogenic potential (Bosch *et al.*, 2006).

2.2.1 Classification of HPVs based on disease association

The difference in the ability of HPVs to cause malignant transformation forms the bases for their classification into Low and High risk types(Georgescu *et al.*, 2018). This idea was conceived from the observation that, some HPV types were more commonly found in cancers than in benign lesions(de Sanjose *et al.*, 2018). HPVs, designated “low-risk” or “non-oncogenic,” such as HPV-6 and HPV-11, induce benign condylomata acuminata and are very rarely found in genital malignancies.(Nicolàs Pàrraga, 2018) These were later followed by a series of studies which established that these risk categories reflect the inherent and differential abilities of the viruses to interfere with the proliferation and

stability of the genome of the affected cells. E6 and E7 proteins from low-risk HPVs, inactivate cellular p53 and pRb tumour suppressor proteins less efficiently than do E6 and E7 proteins from high-risk HPVs (Münger and Howley, 2002).

2.2.2 Epithelial Tropisms

In order for infection to occur, there must be changes in the conformation of the capsid protein(McBride, 2021), which may also influence the tropisms of individual HPV types(Cosper *et al.*, 2021). Therefore, tropisms of the papillomaviruses are thought to be primarily controlled at the level of viral gene expression, where regulatory elements within the Long Control Region (LCR) are an essential determinant (Kranjec *et al.*, 2016; Ina *et al.*, 2021).

Furthermore, all papillomaviruses contain well-conserved core genes involved in replication and packaging with greater diversity in the E6, E7, E5, and E4 genes which have roles in driving cell cycle entry, immune evasion, and virus release (Doorbar *et al.*, 2012) and therefore the tropisms of particular types. Notably, Beta papillomaviruses lack a recognizable E5 ORF, located downstream of E2 in Alpha papillomaviruses, and which along with E6 and E7, is involved in immune evasion and in optimizing genome amplification efficiency. The E4 protein (which plays a role in virus escape from the epithelial surface), like E5, shows considerable sequence heterogeneity between types as well. All this diversity is thought to reflect the different tropisms and transmission routes of different papillomaviruses (DiMaio and Petti, 2013),(Doorbar, 2013) Perhaps more surprisingly, given its importance in genome amplification, the HPV E6 protein is absent

in HPV 101, 103, and 108 (Gamma genera (Nobre *et al.*, 2009),(de Villiers, 2013). Mucosal HPV are a group of HPVs with high affinity for mucous membrane and belong to the alpha genus, including the high–risk HPV types 16 and 18 and are associated with more than 99% of cervical cancers(Gheit, 2019).

Most cutaneous HPV types express E6 and E7 gene products that are structurally similar to those of the mucosal types but their genome does not contain an identifiable E5 Open-Reading Frame (ORF)(Gheit, 2019).Epidemiological studies have clearly demonstrated that a subset of cutaneous HPV types classified into the beta genus are commonly and consistently found in non-melanoma skin cancers. These skin tumours arise mostly at sites exposed to the sun and, contrary to mucosal types, the EV-HPV DNA copy number appears to be much lower than one copy/cell (Asgari et al., 2008).

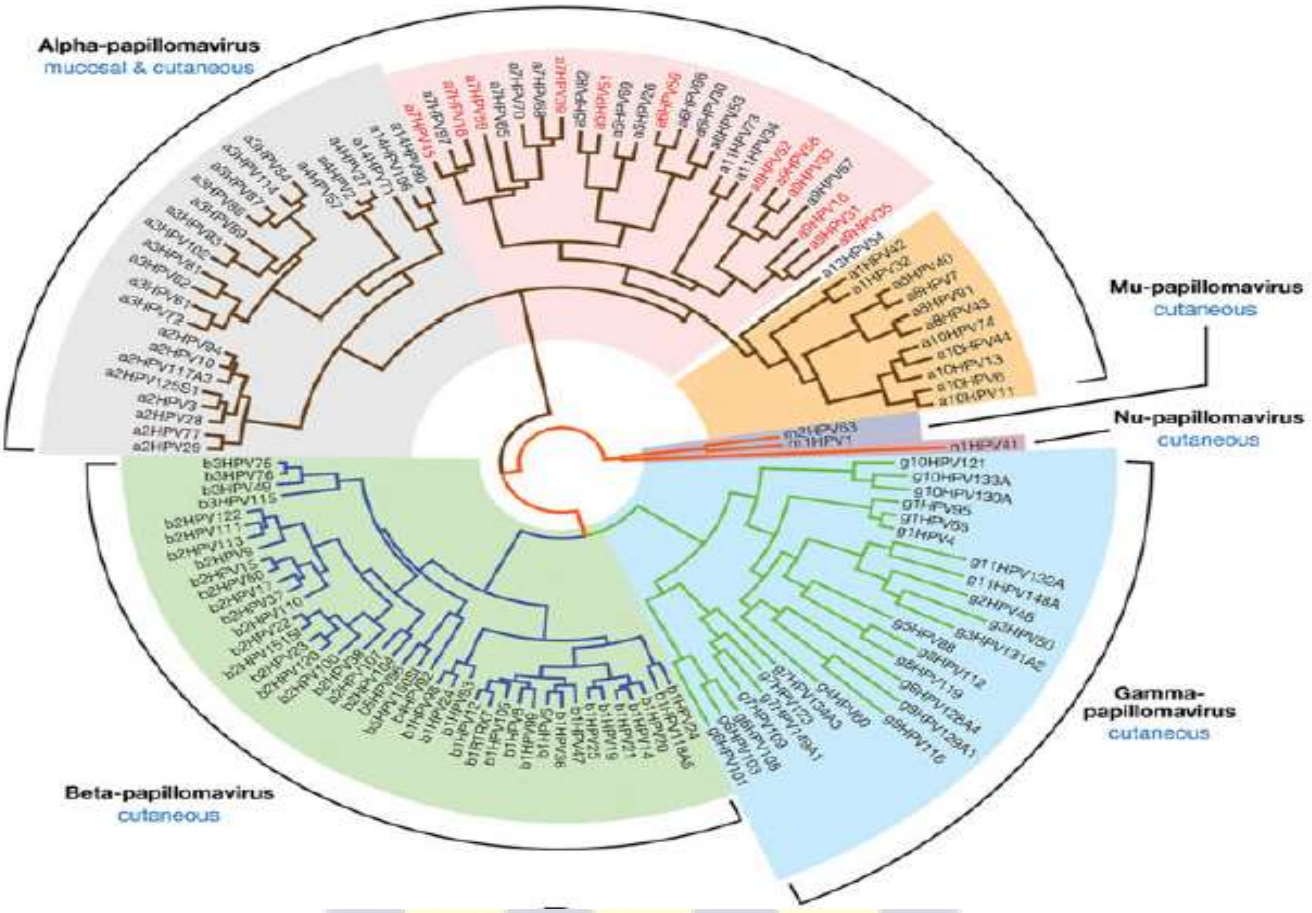

Figure 2.2-1: The human papillomaviruses types found in humans fall into five genera, with the Alpha and the Beta/Gamma genera representing the largest groups. The evolutionary tree is based on alignment of the E1, E2, L1, and L2 genes (Doorbar *et al.*, 2012)

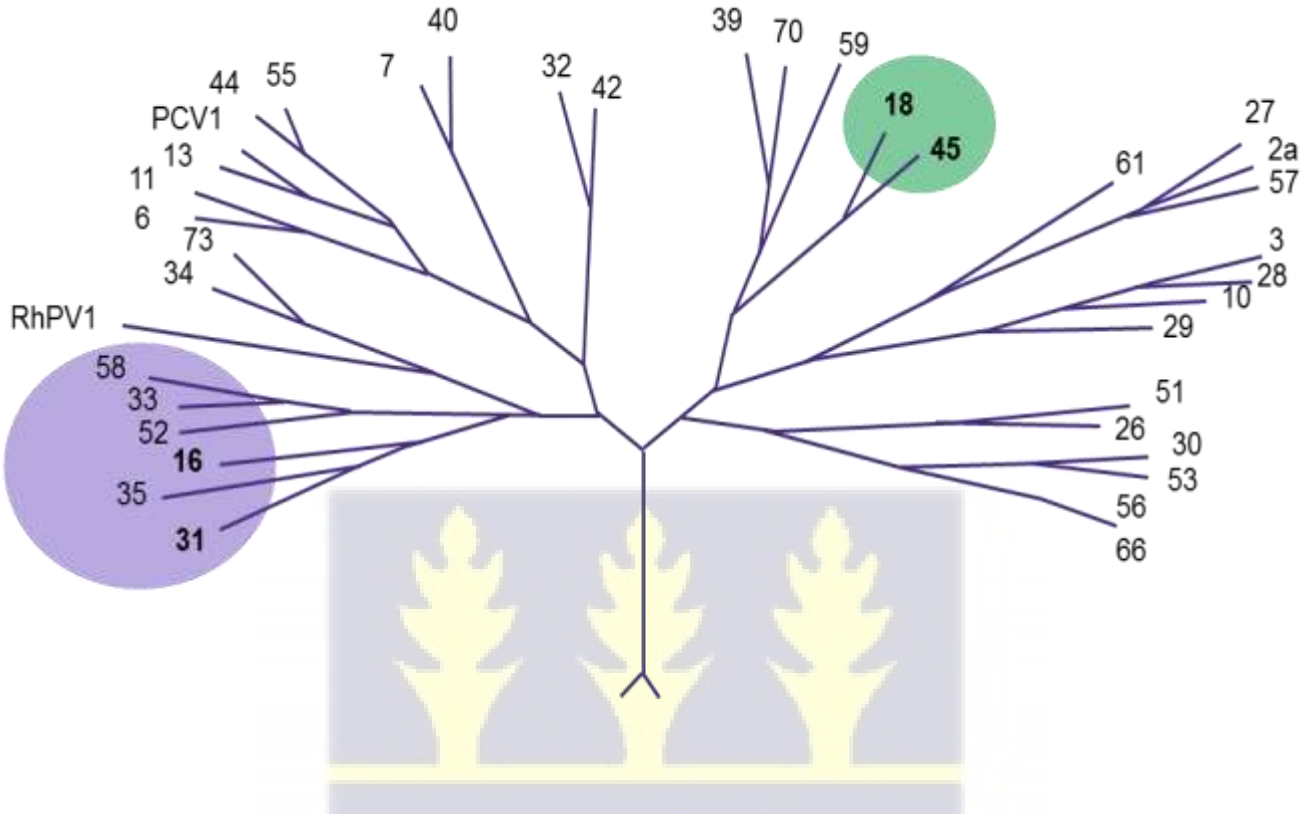

Figure 2.2-2: The alpha-papillomavirus genus of the papillomavirus phylogenetic tree. Oncogenic types closely related to HPV 16 and 18 are highlighted HPV 16 is most closely related to HPV 31. HPV 18 is most closely related to HPV 45. Source: Rose and Stoler, (2006).

2.3 MOLECULAR BIOLOGY OF HUMAN PAPILLOMAVIRUSES (HPV)

Papillomaviruses are diverse and have been found in many different animals as well as in humans and are specific for their respective hosts. More than 200 types of HPV have been identified on the basis of DNA sequence data showing genomic differences(Cullen *et al.*, 2015). They are one of the oldest, largest, and most diverse of the known virus families. HPVs, like all papillomaviruses, target the stratified squamous epithelia of the body(McBride, 2017). Genital Human papillomaviruses (HPVs) are sexually transmitted viruses, the high-risk types are well known to be associated with the development of cancer of the cervix and some cytological precursors which are totally preventable conditions. About 70% of cervical cancer cases are caused by HPV genotypes 16 and 18 and the eight most common genotypes (HPV 16, 18, 31, 33,35,45,52 and 58) contribute to 90% of cervical cancer cases(Zhao *et al.*, 2017).

2.3.1 Organization of the HPV Genome

The HPV genome is functionally recognisable as three regions with the coding potential of about six early genes (E1 to E7; there is no E3), two late genes (L1 and L2) and an upstream regulatory region (URR) or noncoding region (NCR) of about 850 base pairs separating the first two regions(Azzawi, 2013). In general, each open reading frame (ORF) in a papillomavirus genome is often referred to as a gene. However, a gene, in molecular terms, is defined as the entire nucleic acid sequence that is necessary for the synthesis of a functional transcript. According to this definition, a gene is not equivalent to an ORF. In eukaryotes and many viruses, a gene usually contains exons and introns(Sieber *et al.*, 2018). An ORF encoding a polypeptide is usually spread across multiple exons from various parts of the genome which are combined into a full-length ORF through RNA splicing(Dhamija and

Menon, 2018). This is particularly true in papillomaviruses even though only one ORF (E1^E4) in papillomaviruses spans two separate exons.

The three regions in all papillomaviruses are separated by two polyadenylation (pA) sites: early pA (AE) and late pA (AL) sites (Zheng, 2014). The early region of papillomavirus genomes occupies over 50% of the virus genome from its 5′ half and encodes six common open reading frames (E1, E2, E4, E5, E6 and E7) (Doorbar *et al.*, 2012)that translate individual proteins as briefly described below. Two other ORFs, E3 and E8, were also assigned to this region initially, but have now been recanted. The late region of all papillomavirus genomes, covering almost 40% of the virus genome, lies downstream of the early region and encodes L1 and L2 ORFs for translation of a major (L1) and a minor (L2) capsid protein that encapsulate the viral DNA (Zhu *et al.*, 2017) and are useful in the setting of vaccine design (zur Hausen, 2002). All eight viral open reading frames are arrayed in an overlapping linear fashion on one of the double-stranded circular DNA genome. Protein is encoded by both the early and late gene regions, but the URR does not encode (Park *et al.*, 1995). The URR possesses the origin of replication, numerous binding sites for many repressors and activators of transcription including cis-elements required for regulation of gene expression, replication of the genome, and its packaging into virus particles, implying that it may help in defining the hosts for exact HPV types (Turek, 1994). Functionally, the early genes (E1 to E7) are necessary for the replication of the viral DNA and for the assembly of newly produced virus particles within the infected cells. The late genes are expressed just before the egress of virions. Notably, the viral genome does not encode a unique DNA polymerase, which is required for DNA replication, and therefore the virus must depend on

the replication machinery of the host-cell for the generation of viral progeny (Munoz *et al.*, 1996).

2.3.2 Structure of Human Papillomaviruses

Despite the different diseases they cause, HPVs are small, rounded, non-enveloped DNA tumour viruses with a common non-enveloped icosahedral structure, normally between 50-60 nm in diameter (Zheng and Baker, 2006). The HPV virion consists of a single molecule of circular, double-stranded DNA about 8 kilobase pairs in length that is bound to cellular histones and contained within a symmetric icosahedral protein coat, the capsid, which is made by the spontaneous assembly of the L1 major and L2 minor capsid proteins (Winer et al., 2003). It is important to note that HPV virus-like particles (VLPs) can be produced by the expression of L1, alone or in combination with L2, in mammalian or non-mammalian expression systems.

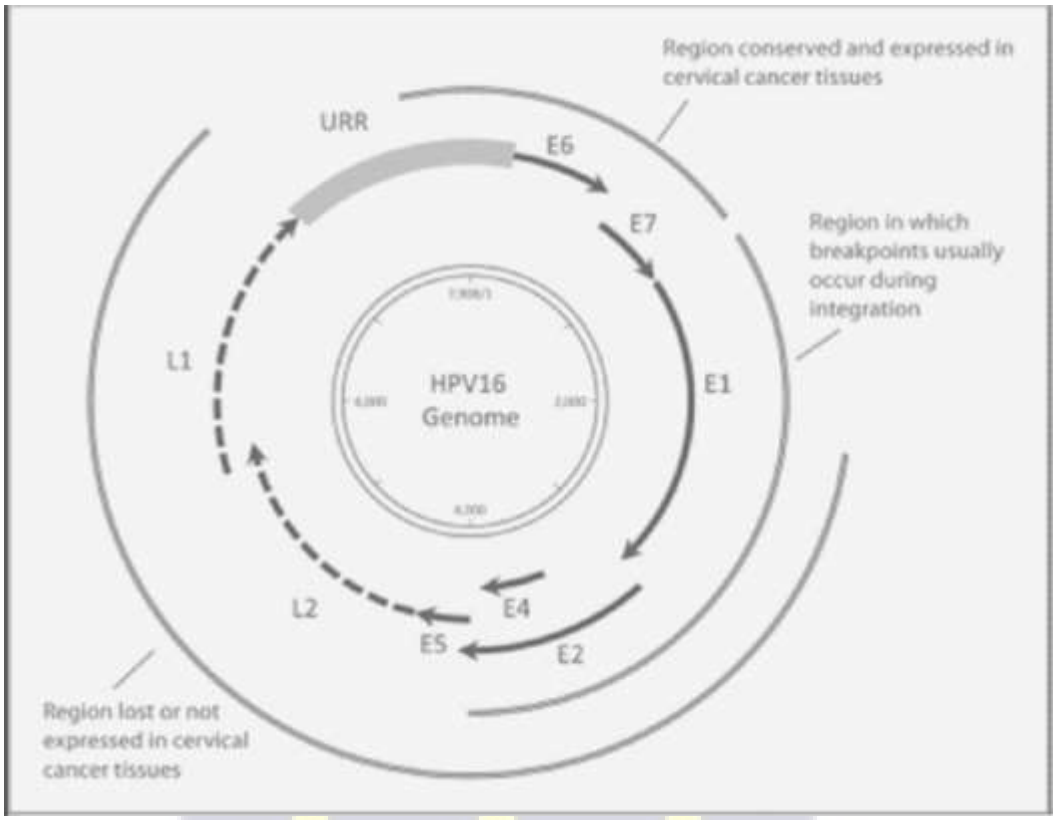

Figure 2.3-1: A schematic diagram of a representative HPV 16 genome. It has three main regions namely, an upstream regulatory region (URR), an early region, which contain open reading frames (ORFs;e.g E1, E2, E3, E4,E5,E6 and E7). (Shah *et al.*, 2020)

Their genomes are made up of double stranded circles (episomes) of close to 8000 base pairs, which contain eight or nine Open Reading Frames (ORFs). Although gene number is limited by the small size of the papillomavirus genome, the number of encoded protein is much greater, as gene expression involves the use of multiple promoters and complex pattern of splicing (Zheng & Baker, 2006)(Shah *et al.*, 2020). The virus coat contains 360 molecules of L1 proteins organized into 72 capsomers, each containing of 5L1 molecules (Schiller & Lowy, 2012). HPV particles also contain different number of L2 molecules which are not fully exposed on the surface of the virion (Rubio et al., 2011). During

infection, L2 becomes available for binding to the extracellular matrix and is cleaved by furin (Kines, Thompson, Lowy, Schiller, & Day, 2009). The main exposed surface region of L1 are made up of a series of hypervariable amino acid loops that are unique between different papillomavirus types, in response to host immune selection pressure with antibodies raised to one HPV type binding to distantly related types occurs only poorly. The virus genome also encodes regulatory proteins that stimulate cell cycle entry and cell proliferation as well as proteins that mediate virus genome replication and transmission. Many of these genes are found within the early region of the virus but the L2 gene product also has key immediate early function in viral genome delivery within the cell (Wang & Roden, 2013).

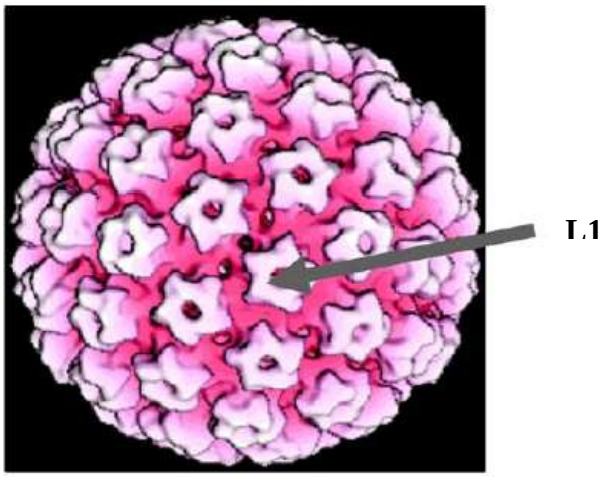

Figure 2.3-2: A model of the virus capsid depicting superficial pentamers. These conformational epitopes on the major coat or capsid protein L1 displayed on the outer surface of the intact virus particle can be recognized by the immune system and forms the basis of HPV vaccines. Source: Stanley *et al.*, 2006.

2.4 SOME DISEASES ASSOCIATED WITH MUCOSAL HPV IN HIV PATIENTS

HPVs are highly ubiquitous and also distributed throughout the body, but with different anatomic predilections that allow three major groups of HPV-associated diseases to be distinguished: (1) cutaneous warts, such as plantar, common, and flat warts; (2) epidermodysplasia verruciformis, a rare autosomal recessive genetic hereditary skin disorder in which the affected individual is abnormally sensitive to HPV (mostly 5 and 8) and has a high-risk of carcinoma of the skin; and (3) genital or mucosal lesions including conditions such as genital warts, laryngeal papillomas, as well as pre-cancers (dysplasias or intraepithelial neoplasias) and cancers of the uterine cervix, vagina, vulva, penis, anus, and of the head and neck region (Bosch *et al.*, 2006).

2.4.1 Benign HPV-associated cervical lesions

Condyloma acuminatum or cervical warts results when HPV infect the ano-genital region with low-risk HPV types such as HPV 6 and 11, although many other genotypes can also be found, including HPV 2, 16, 18, 30–33, 35, 39, 41–45, 51–56, and 59 (McKee *et al.*, 1981; Clifford *et al.*, 2005a; Petry *et al.*, 2012). They present as papules, nodules or soft, filiform, pinkish, sessile or pedunculated growths. In men, genital condylomas more commonly involve the coronal sulcus, the glands penis, and the penile shaft. In women, lesions commonly affect the external genitalia and the cervix (Chuang, 1987) They may be diagnosed by cytologic evaluation of a Papanicolaou (Pap) smear or at the time of colposcopic evaluation for an abnormal Pap smear. The subtle appearance of thickened, raised, and whitish epithelium can be easily concealed by cervical mucus (Bosch *et al.*, 2006). These warty lesions may present at multiple sites, affecting not only the external cervix (exocervix), but potentially extending up

into the cervical canal to the squamo-columnar junction (Bosch *et al.*, 2006). The HPV types that cause benign genital warts can also cause problematic papillomas at oral sites, which can be difficult to treat because of their location.

Focal epithelial hyperplasia is a rare HPV-related disease of the oral mucosa that is more common in children and women. Lesions are mainly located in the lower lip, but less frequently may affect the upper lip, tongue, oral mucosa, oropharynx, palate, and floor of mouth. HPV 13 and 32 are the most common cause (Vera-Iglesias *et al.*, 2007).

2.4.2 Pre-malignant HPV-associated lesions

Pre-cancerous lesions are defined biologically as lesions that have, in principle, a capacity to progress potentially to invasive cervical cancer if left untreated (Cuschieri *et al.*, 2004). The pre-malignant changes characteristic of cervical cancer represent a spectrum of histological abnormalities ranging from low-grade squamous intraepithelial lesion (LSIL)/CIN1 through high-grade squamous intraepithelial lesion (HSIL)/CIN2 to HSIL/CIN3 carcinoma-in-situ (Woodman *et al.*, 2007). The severity of neoplasia reflects the extent to which basal-like cells (i.e. poorly differentiated cells with a high nuclear/cytoplasmic ratio) extend toward the epithelial surface and the extent of suprabasal cell division. Low-grade lesions typically show evidence of productive viral infection with the presence of koilocytes in the suprabasal cell layers being regarded as a key manifestation of CIN1/LSIL. HPV is detectable in 90–100% of cervical abnormalities, ranging from incipient cytological abnormalities and dysplasia (Bedoui *et al.*, 2009)to cervical cancer (Solomon *et al.*, 2002; Jenkins, 2007)[50–(Muñoz *et al.*, 2003)

HSILs are mostly found in association with HR-HPV while LSILs are frequently associated with LR-HPV-6 and -11 (Lacey *et al.*, 2006).

2.4.3 Cervical Cancer

It is now universally acknowledged that malignant squamous and glandular neoplasms of the cervix are caused by infection of cervical epithelium by specific HPV types (Bosch *et al.*, 1995a; Munoz *et al.*, 2003a). Persistent intraepithelial neoplasias may eventually evolve into invasive carcinoma (Cuschieri *et al.*, 2004). Almost 90% of cervical cancers are squamous cell carcinomas. The fraction of cervical cancer attributable to HPV-16 is 53.5% (Munoz *et al.*, 2004). Taken together, HPV-16 and HPV-18 account for about 70% of all cervical squamous cell carcinomas. Furthermore, the projected HPV-16/18 fraction is higher in more advanced (72–77%) than in less advanced (65–72%) regions. Approximately 41–67% of high-grade squamous intraepithelial lesion (HSIL), 16–32% of low-grade squamous intraepithelial lesion (LSIL) and 6–27% of atypical squamous cells of undetermined significance (ASCUS) are also projected to be HPV-16/18-positive (Munoz *et al.*, 2004). Taken together, HPV is responsible for 5% of all human cancers, over 10% of cancers in women and up to 15% of cancers in women in developing countries (Winer and Koutsky, 2004).

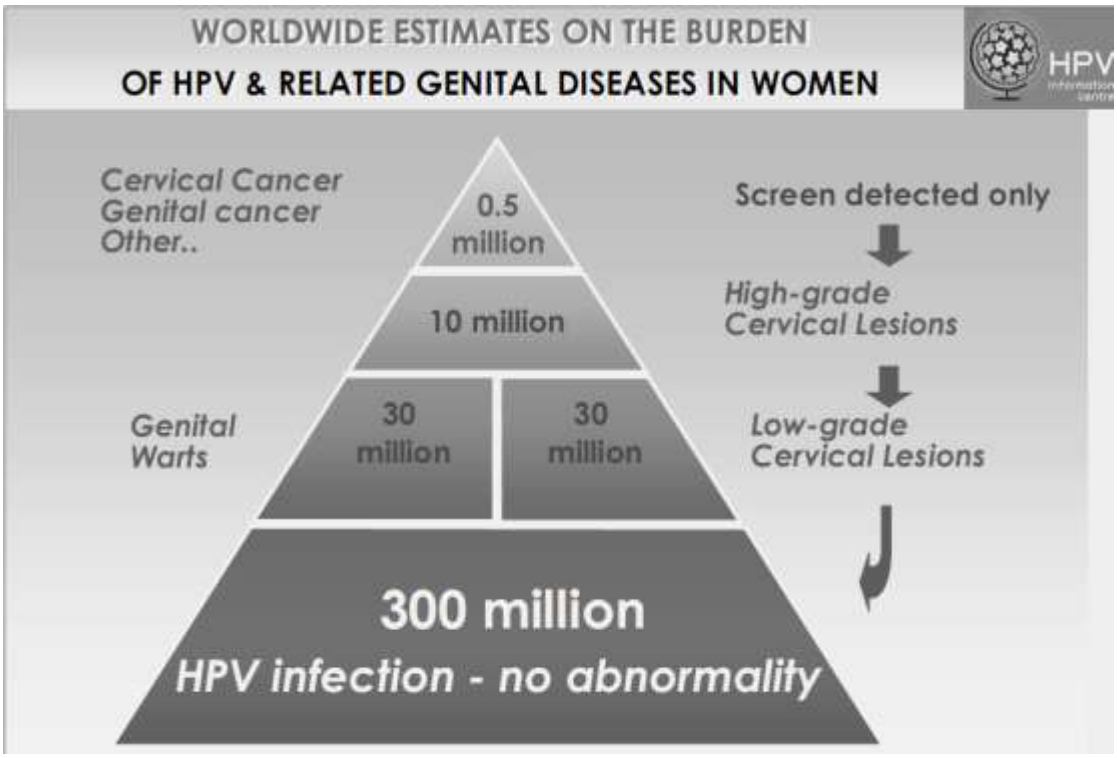

Figure 2.4-1: Estimates of the global burden of HPV related diseases in women. The graph shows on the left hand the clinically apparent conditions related to HPV infections. The right hand side shows the diagnoses that occur in populations where screening is used. The body of the pyramid shows current estimates of the worldwide number of cases, rounded for convenience. Adapted from (WHO/ICO, 2010a).

2.4.4 Other anogenital cancers

These include cancers of the vulva, vagina, penis, and anus. Most vulvar cancers (92%) are solitary, keratinizing squamous cell carcinomas (SCC). HPV prevalence is 90% in vulvar intraepithelial neoplasia and basaloid or warty cancers, but is found in only 6% of keratinizing SCC (Hording *et al.*, 1996) (Toki *et al.*, 1991). HPV 16 is the most prominent type in vulvar cancer, with HPV 18, 21, 31, 33, and 34 detected at lower frequencies. In addition, HPV is responsible for 85% of vaginal cancer, with HPV 16 being detected in 60% of invasive tumors.

HPV is also detected in basaloid and warty cancers of the penis, but only rarely in keratinizing SCC and verrucous cancers of the penis. In invasive penile cancer, HPV 16 is the most prevalent type (40–70%), followed by HPV 6 (22%), 52 (15%), and 11 (4%) (Rubin *et al.*, 2001). HPV is present in 80–96% of anal cancer with HPV 16 being the most prevalent type (De Vuyst *et al.*, 2009) Anal cancer is more common in men who have sex with men, individuals with a history of anal warts, and in immunosuppressed populations.

2.4.5 Head and neck cancer

HPV is recognized as a major risk factor for the development of head and neck squamous cell cancers (HNSCC). A recent meta-analysis showed that HPV prevalence in HNSCC increased significantly from 41% in 2000 to 72% in 2004 (Mehanna *et al.*, 2013). HPV prevalence is significantly higher in oropharynx SCC than in the oral cavity with the tonsil having higher prevalence than other anatomic sites (Herrero *et al.*, 2003). These HPV-associated cancers display clinical and molecular features distinct from other HNSCCs. The patients with HPV-positive cancer have at least a 50% improvement in overall survival at 5 years, which is equivalent to an approximate 30% difference in absolute survival. HPV association is now part of routine diagnostic procedures when assessing the prognosis of HNSCC. HPV 16 is the most common type found in HNSCC, but other HPV types such as 18, 31, 33, and 35 can also be detected (Mehanna *et al.*, 2013; Awuah, 2014).

2.5 EPIDEMIOLOGY OF HPV INFECTION

HPV, the most common infection acquired sexually contribute to approximately 5% of all human cancers and 12% of all female cancers (zur Hausen, 2002). With improving methods of detection, HPV DNA has been found in almost all cervical cancer cases worldwide (Bosch *et al.*, 1995b; Walboomers *et al.*, 1999). In addition to cervical cancer, high-risk HPV infections of the penis, vulva, and vagina can lead to cancer at these sites. Though these cancers are not as common as cervical cancer in developing countries, an estimated 85% of anal cancers, 50% of the cancers of the vulva, vagina, and penis, 20% of oropharyngeal cancers, and 10% of laryngeal and oesophageal cancers are attributable to HPV (zur Hausen, 1996; WHO, 1999).

Numerous studies have stated the prevalence of HPV DNA in both ambiguous and negative cytologic results as well as in cancer (IARC, 1995; Gjooen *et al.*, 1996; Ferenczy *et al.*, 1997; Bosch and de Sanjose, 2003; Baseman and Koutsky, 2005; Herrero *et al.*, 2005; Domfeh *et al.*, 2008) . Crude prevalence estimates of HPV infection (based on HPV testing of asymptomatic women in the general population) range from 2 to 44%. This wide variation in prevalence estimates could also be explained by age differences among population samples studied, and by differences in the molecular sensitivity of the various HPV DNA assays used to detect viral DNA (Bosch and de Sanjose, 2003).

The designated "high-risk" group comprises fifteen members (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) (Munoz *et al.*, 2003b). High-risk HPV DNA has been shown to be present in (Bosch *et al.*, 1995b; Walboomers *et al.*, 1999) and implicated in the

development of cervical pre-malignant lesions and invasive cervical cancer worldwide (Kjaer *et al.*, 1996; Liaw *et al.*, 1999; Nobbenhuis *et al.*, 1999; Wallin *et al.*, 1999). Recent evidence suggests that so-called high-risk types account for almost 90% of cervical infections (Aral and Holmes, 1999). Twelve are classified as "low-risk" types (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108) and have the least propensity to persist and induce malignant lesions (Munoz *et al.*, 2003b). Three are classified as "probable high-risk" types (26, 53, and 66) (Munoz *et al.*, 2003a) being less frequently found in cancers but are often found in squamous intraepithelial lesions (SILs). Some authors refer to these HPV types as intermediate-risk (Burd, 2003a).

2.5.1 Global Prevalence of HPV DNA

Genital HPV infection is the most common STI among women (Coutlee *et al.*, 2002). An estimated 291 million women are expected to be infected with HPV-DNA worldwide. Out of this, approximately 105 million women worldwide will have an HPV-16 or -18 infection at least once in their lifetime (Burchell *et al.*, 2006). There have been studies on the prevalence of HPV DNA in cervical specimens from women with all possible cytological and histological outcomes. The diversities in methodology limit comparability of findings from these studies. In this regard, the International Agency for Research on Cancer's (IARC) data from 15 areas in 4 continents regarding women aged 15 to 74 years provides a suitable reference point for HPV epidemiology. In this large-scale study, the age-standardized prevalence ranged from less than 5% in some Mediterranean and South East Asian countries to more than 15% in several countries in Latin America and among a few African populations (Clifford *et al.*, 2005b). In another comprehensive review of studies that used standardized inclusion criteria

and controlled for variables that may have challenged the comparability of the studies, prevalence estimates of HPV infection among women with negative cytology results ranged from 10% to 15%. Age-specific prevalence estimates showed HPV DNA to be more prevalent among young women, with a decline in young adult women and a variable pattern afterwards (Kjaer *et al.*, 2001). In some countries, notably in the Americas, the prevalence increased again in postmenopausal age groups. In Europe, a plateau in the middle-age groups was maintained whereas in other high-prevalence countries in Asia and Africa the prevalence remained fairly constant across all age groups (Kjaer *et al.*, 2001). A meta-analysis of 78 published studies estimated HPV prevalence among women with normal cytology (Gellin *et al.*, 2000; Womack *et al.*, 2000). The adjusted global prevalence of 10.41% (95% confidence interval, CI: 10.2–10.7%) compares well with the IARC finding, with considerable variation by region. Only 8 out of the total 78 studies came from Africa, representing 4% of the total number of women tested. Africa had the highest age-adjusted prevalence of 22.12%. The WHO estimates that 21.5% of women from Western Africa have an infection at a given time (WHO/ICO, 2010a).

2.5.2 Prevalence of HPV Genotypes in Cervical Cancer

A collective look at 12 studies carried out across 25 countries using standardized protocol and GP5+/6+ HPV-DNA testing by means of PCR assessed HPV prevalence in 3085 women with cancer of the cervix (Munoz *et al.*, 2004). The general HPV-DNA occurrence was 96% and the 15 common types observed, in descending order were, HPV-16, -18, -45, -31, -33, -52, -58, -35, -59, -56, -39, -51, -73, -68 and -66. HPV-16 and -18 accounted for 70% and the eight most found types (HPV-16, -18, -45, -31, -33, -52, -58 and 35) which contributed to 89% of all cervical cancer cases worldwide (Clifford *et al.*, 2006a). A greater prevalence of HPV-16

was observed in Northern Africa, Europe and North America, of HPV type 45 was observed in sub-Saharan Africa and of HPV type 31 in Latin America (Munoz *et al.*, 2004). A complete meta-analysis of greater than 14,500 cases from studies put out up to January 2006 has been done (Curtis and Sutherland, 2004) the result was similar the results of the pooled study. The common HPV types seen were, HPV-16, -18, -31, -33, -35,-45, -52, and -58. HPV-16 prevalence was different from 52% in Asia to 58% in Europe, and HPV-18 was not the same 13% in South/Central America to 22% in North America (Curtis and Sutherland, 2004).

2.5.3 Transmission and Natural History of Cervical HPV Infection

HPV is the most common sexually transmitted disease among women (Coutlee *et al.*, 2002). Available experimental evidence suggests that a cervical HPV infection, as distinguished from HPV infections at more superficial sites which may follow vertical or horizontal transmission, is only acquired as a result of penetrative vaginal sexual intercourse (Ley *et al.*, 1991; Koch *et al.*, 1997; Quint *et al.*, 2012). Barnabas and colleagues (de Sanjose *et al.*, 2007) estimated the probability of male-to-female transmission for HPV-16 as 60% per partner making it more transmissible than other viral STIs but comparable to bacterial STIs (Fleury *et al.*, 2008) such as gonorrhoea (50%) and syphilis (60%) (Cutts *et al.*, 2007).

In order for the HPV to be able to enter and start an infectious process there is a requirement for a loss of continuity of the epithelium, so that the virus can be in contact with the permissive cells, which are the basal cells of the epithelium.(Miller and Torday, 2017) Once they have infected the target cells, viral replication starts. The assembly of the virions is carried out in

the upper strata of the epithelium when the cells have differentiated (granular cells), since this is a prerequisite for this maturity event and cell differentiation.

During sexual activity, micro-trauma of the genital epithelium, particularly in the transformation areas of the cervical epithelium, allows exposure of the basal cells to active proliferation of the various types of HPV, allowing union between the receptor of the basal cells with the protein from the viral capsid L1. Once the virus is joined to the cell surface, it beds into the host cell cytoplasm, a process that has been identified as endocytosis (Bousarghin *et al.*, 2003). Cervical HPV infection is usually only probable after most women begin their first sexual relationship (Collins *et al.*, 2002). Due to the usually transient nature of infection, the virus might be detected only intermittently and the concurrent or sequential detection of different HPV types is common (Ho *et al.*, 1995). The median duration of a newly detected cervical HPV infection is reported to be approximately 9 to 12 months, with only 10% of infections still detectable after 24 months (Woodman *et al.*, 2001). In practice, it must be noted that there is often an overestimation of the median duration of infection since infections clear quickly (e.g.,<3–4 months) and escape most study designs (Ho *et al.*, 1995). HR-HPV infections, particularly HPV 16, infections tend to last longer than those of LR-HPV types (Castellsague *et al.*, 2001; Woodman *et al.*, 2001).

2.5.3.1 Multiple Infections

Co-infection with multiple HPV types is found in 20% to 50% of HPV-infected women (Liaw *et al.*, 2001; Clifford *et al.*, 2005b) and is currently arousing much research interest. Although the tendency of multiple HPV types to cluster within women has been frequently observed

(Thomas *et al.*, 2000; Liaw *et al.*, 2001; Rousseau *et al.*, 2001; Mendez *et al.*, 2005), just 1 out of 12 designated carcinogenic variants must necessarily be present to cause cervical cancer (Walboomers *et al.*, 1999).

Results from longitudinal studies in HPV-co-infected women propose that the existence of more than one HPV genotypes does not support persistence of HPV infections (Molano *et al.*, 2003; Plummer *et al.*, 2007). It is believed that the excess infections are independent events occurring because all HPV infections generally share the same transmission route and are all associated with the same risk factors (Thomas *et al.*, 2000; Mendez *et al.*, 2005).

2.6 EPIDEMIOLOGY OF CERVICAL CANCER

2.6.1 Globally and in Africa

World-wide, cervical cancer is the second commonest cause of female cancer after breast cancer, accounting for up 13% of all female cancers. Each year, across the world, approximately 490,000 women are newly diagnosed and 274,000 die from invasive cancer of the uterine cervix induced by oncogenic types of human papillomavirus (HPV) (Franceschi *et al.*, 2006; Ginsburg *et al.*, 2017) The overwhelming majority of women affected by this completely preventable disease live in resource-constrained nations where access to screening services and health insurance coverage is limited or non-existent (Franceschi et al., 2006; Louie, De Sanjose, & Mayaud, 2009; Wabinga et al., 2014). In the so called *developed countries* less than 0.5% of women will develop cervical cancer before age 65 but in *developing countries*, 2% of women are likely to get it before age 65 (Parkin and Bray, 2006;

WHO/ICO, 2010a). The highest number of cases per region is reported in sub-Saharan Africa, South America, South-Central Asia and South-Eastern Asia (Parkin and Bray, 2006). In West Africa, estimated age-standardized rates per 100,000 women per year range from 26.9 in Cote D'Ivoire, through 28.6 in Burkina Faso, 30.0 in Togo, 33.0 in Nigeria, 39.5 in Ghana to 56.3 in Guinea as compared to 15.2 in the world (Figure 2.6-1) (WHO/ICO, 2010a).

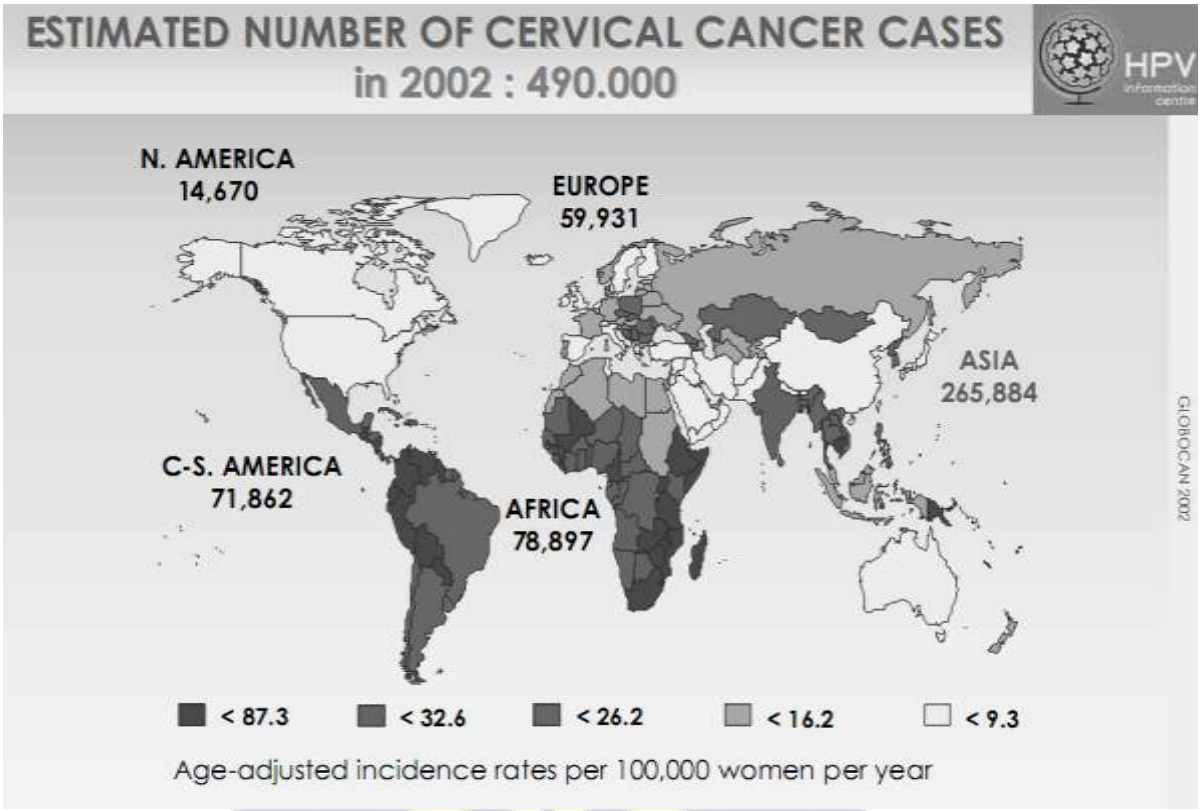

Figure 2.6-1: Global estimates of cervical cancer incidence. The highest adjusted incidence rates occur in Sub-Saharan Africa where fewer studies have been carried out compared to most of Europe and America which have recorded the least number of cases. Adapted from (WHO/ICO, 2010a).

Mortality rates are substantially lower than incidence. Worldwide, the ratio of mortality to incidence is 55% (Parkin and Bray, 2006). Because cancer of the cervix is found cervical young women, it is a leading cause of years of life lost (YLL). In Latin America, the Caribbean and Eastern Europe, cervical cancer contributes greatly to YLL than other diseases such as tuberculosis, maternal conditions or AIDS. It also makes the major input to YLL from cancer in the highly populated regions of sub-Saharan Africa and South-Central Asia (Parkin and Bray, 2006).

2.6.2 Ghana

Women aged 15 years and above make up 6.57 million of Ghana's population and this age group can get cervical cancer (WHO/ICO, 2010a). There is little information on the actual prevalence and incidence of cervical cancer in Ghana owing to lack of a national population-based cancer registry. The country has several cancer registries which are hospital-based data collected from hospital cases. It is estimated that Ghana has one of the highest rates in the world being a third world country with no organized cervical cancer prevention programme (Parkin *et al.*, 2003; Parkin and Bray, 2006). In one hospital-based study, cervical cancer accounted for 58.3% of all gynaecologic cancers (Agorastos *et al.*, 2005). In 2006, Wiredu and Armah (2006) reported a hospital based review of human tisues and mortality records of a 10 year period in which cervical cancer was the fourth most common cause of cancer

mortality in females after malignancies of the breast (17.24%), haematopoietic organs (14.69%), and liver (10.97%). Based on data collected elsewhere in sub-Saharan Africa, the WHO projected that cervical was the leading cause of female mortality among the cancers with over 2,000 deaths in the year 2010 alone (IARC and WHO, 2008; WHO/ICO, 2010a).

The Kumasi Cancer Registry was established in Ghana in 2012 to provide information on cancer cases seen in the city of Kumasi. Recently, the registry has published data from the year 2012. Among females, the most common cancers were breast cancer (33.9%), cervical cancer (29.4%), ovarian cancer (11.3%) and cancer of the Endometrium (4.5%) (Laryea *et al.*, 2014).

2.7 EPIDEMIOLOGY OF HIV/AIDS IN GHANA

The HIV epidemic in Ghana is described as a widespread epidemic with constant prevalence of more than 1%in the general population (Ghana, 2012) Using the annual sentinel surveys carried out among antenatal attendants, the HIV epidemic in Ghana seems to have a downward trend, from 3.6% in 2003 through to 2.2% in 2008 and to 2.0% in 2011(Ampofo, 2009; Agyei-Mensah and Aikins, 2010). The estimated deaths that occur annually for 2010 and 2011 were 17,230 and 15,263 respectively. Sentinel survey data for 2012 gave a prevalence of 1.7% and 11,655 AIDS death. In 2013 sentinel report, the prevalence of HIV further decreased to 1.3% with an estimated death of 10,074.Recent data from the 2014, 2015 and 2016 sentinel reports depicts an upsurge of HIV prevalence. The HIV prevalence rate for2014 was 1.6% (up from 1.3% in 2013), then went up to 1.8% in 2015 and then 2.4% in 2016

2.8 HUMAN PAPILLOMAVIRUSES AND HIV/AIDS

2.8.1 Effect of HIV infection on HPV Acquisition

Human papillomavirus (HPV) is the most common sexually transmitted infection and at least 50% of sexually active people will get HPV infection at some time in their lives (Bernard *et al.*, 2010). HPV infection can lead to genital warts and (pre)malignant lesions. It is estimated that 80% of women get infected by hrHPV in their lifetime. However, most of these infections are sub-clinical or transitional and in normally cleared after 12-30 months in 70-90% of infected women (Alba, Cararach, & Rodríguez-Cerdeira, 2009). For both hrHPV and low risk viruses, there are reports that the infection lasts for about 4-5 months (Alba et al., 2009). Though majority of infections can be cleared within 2 years by the human immune system, some infections persist in infected cells and for people with a compromised immune system this leads to a rapid transformation of the infected cells to precancerous and cancerous lesions (Hawkins et al., 2013).

A number of studies have shown that HIV-infected women are at a higher risk for HPV infection and progression of HPV lesions to cervical cancer compared to HIV-negative women (Palefsky *et al.*, 1999);Banura et al., 2011). Underlying this observation is that HIV infected women seem to have a higher HPV DNA persistence rate than HIV negative women (Banura et al., 2010) and are also infected with multiple HPV types than HIV-negative women (Chakravarty et al., 2016; Williamson, 2015). In a study carried out in South Africa, a high prevalence of multiple HPV infections was observed (Mbulawa, Coetzee, & Williamson, 2015). Invasive cervical cancer samples were found to have far more multiple HPV infections

(PR =1.75, 95% confidence intervals: 1.18 to 2.58) in a meta-analysis carried out among women infected with HIV in Africa (Clifford et al., 2016).

In a recent meta-analysis, it was found that women living with HIV have a higher chance of getting infected with the human papillomavirus (Liu, Sharma, Tan, & Barnabas, 2018). In cross-sectional studies HIV seropositivity is associated with increased prevalence of cervical HPV infection, greater prevalence of precancerous cervical lesions, and with grade of dysplasia. Prospective studies have demonstrated that low CD4+ T-cell count and/or high HIV viral load are associated with the incident detection and duration of HPV infection. A review of available literature on the HPV type-specific risk among HIV-seropositive women (Clifford et al., 2006b) revealed that the proportion of HIV-positive women with HPV16 rose with increasing severity of cervical lesions. Nevertheless, HPV16 remained underrepresented in HIV positive women with HSIL, who were significantly more likely to be infected with HPV types 11, 18, 33, 51, 52, 53, 58 and 61 compared with the general female population with HSIL. Regardless of the type of treatment, there is a higher failure rate for cervical neoplasia in women with HIV, although the subsequent lesions are typically low grade. The rationale for treatment is to eliminate pre-cancerous lesions and ensure the lowest possible risk of cancer through constant surveillance. In addition, the large numbers of individuals infected with other HR-HPV genotypes (Banura et al., 2011) suggests that these other HR-HPV types should be considered in the development of the next generation of vaccines.

2.8.2 Prevalence of HPV in HIV infected women

The prevalence of HPV among women living with HIV varies between parts of the globe, 80% in Zambia and 90-100% in Uganda (Banura et al., 2011). In another related study carried out in Brazil, an HPV prevalence of 65.6% was observed among HIV positive men undergoing antiretroviral treatment (Guimarães *et al.*, 2011). A prevalence of 64% was observed in a meta-analysis carried out on women living with HIV in Kenya (Menon *et al.*, 2016).

It is reported that, HIV infection increases the prevalence of HPV among women (Liu, Sharma, Tan, & Barnabas, 2018). In spite of the variation in prevalence of human papillomavirus infection among women living with HIV from different parts of the globe, some reports indicate a twofold higher prevalence equalled to their HIV negative counterparts. Sub-Saharan Africa reported 62% HPV prevalence compared to 24%, 30% compared to 5% in North America and 30% compared to 14% in Europe, in HIV positive and HIV negative women respectively (Bruni *et al.*, 2010; Clifford *et al.*, 2017). In a study carried out in India, a prevalence of 32.2% HPV infection was observed among HIV positive women compared to 9.1% among HIV negative women (Sarkar *et al.*, 2011). A meta-analysis of data from United States of America showed HPV prevalence of 46% among women living with HIV compared to 29% in women from the general population (Park *et al.*, 2016). In a study carried out in Cape Town on 1371 HIV-negative women and 8050 HIV-negative women, there was an increase in the prevalence of high-risk HPV (HR-HPV) among HIV positive women (52.4%) than among HIV negative women (20.8%) (McDonald *et al.*, 2014).

2.8.1 HIV and HPV type distribution

HIV infection is a strong independent risk factor for cervical cancer (Schlecht et al., 2001) and it is not surprising that the association between cervical cancer and HIV infection is reflected by a corresponding correlation between HPV infection and HIV infection (Ferenczy et al., 2003). Actually the high incidence of cervical cancer among HIV positive women may be explained by increased HPV persistence (Minkoff *et al.*, 1998; Ahdieh *et al.*, 2001; Moscicki *et al.*, 2004), susceptibility to a broad range of HPV genotypes (Goncalves *et al.*, 1999; Baay *et al.*, 2004; Chaturvedi *et al.*, 2005), especially the highly oncogenic types 16 and 18, and to infection with multiple HPV types (Sun et al., 1997). A review of available literature on the HPV type-specific risk among HIV-seropositive women revealed that the proportion of HIV-positive women with HPV16 rose with increasing severity of cervical lesions. There is mounting evidence that other HPV types such as HPV 33, 35, 45, 52 and 58 can be more prevalent than HPV 16 and 18 in some African countries (Munoz *et al.*, 2004). For example, the proportion of HPV infection caused by HPV-16/18 was lower in HIV-positive women in Cameroon after HPVs 45, 58 (Desruisseau *et al.*, 2009). Also, HPV 35 was slightly more common than HPV 16 in Mozambique both in women with normal cytology and in those with HSIL or worse (Castellsague et al., 2001). HPV 52 was found slightly more frequently than HPV 16 or HPV 35 in Kenya (De Vuyst et al., 2003), in Zambia (Sahasrabuddhe *et al.*, 2007), in Burkina Faso (Didelot-Rousseau *et al.*, 2006) in colposcopically normal women in Zimbabwe (Gravitt et al., 2002) and in women presenting to a sexually transmitted infections clinic in Uganda (Blossom *et al.*, 2007). In Senegal, HPV

16 and 58 were the most common types overall and in women with cervical lesions (Xi et al., 2003).

2.8.2 Persistence of HPV in HIV Positive Women

Aside increasing prevalence, immunodeficiency has also been a major cause of HPV persistence among both males and females worldwide mostly as a result of HIV infection A study carried out in Nigeria established that, HIV positive women have higher persistence of HPV infection than their HIV negative counterparts (Adebamowo *et al.*, 2016; Adebamowo *et al.*, 2017){Gupta, 2015 #1248 A seven-fold increase in the rate of persistence of human papillomavirus infection was observed over a twelve-month period in a cohort of HIV infected young women in South Africa (Adler et al., 2015). A couple of other studies have also established that there is evidence of persistence of HPV infection among women living with HIV (Kelly et al., 2017){Miranda, 2013 #1261}(Dalstein *et al.*, 2003; Lörincz and Richart, 2003; Cogliano *et al.*, 2005; Kriek *et al.*, 2016).

2.8.3 HIV and Cervical Cancer

Cervical cancer is the major disease caused by human papillomavirus infection in women living with HIV. A recent systematic review and meta-analysis showed that, HIV positive women had increased incidence of Low-grade squamous intraepithelial lesions (LSIL) and high-grade squamous intraepithelial lesions (HSIL) which was largely due to increased HPV persistence(Liu et al., 2018) The odds of having CIN2+ was 6.55 times (95% CI 4.44-9.67) greater in HIV positive women was observed in a study in Malawi (Kohler et al., 2016). In

another study, increased cervical cancer was documented among HIV positive women than in the general population with standardized incidence ratios (SIRs) of 4.2 to 8.9 (Chaturvedi, Madeleine, Biggar, & Engels, 2009)

2.9 PREVENTION OF CERVICAL DISEASE IN HIV

2.9.1 Primary Prevention with Available Prophylactic Vaccines

It is well understood that population-based prophylactic immunization based on an efficacious vaccine could potentially prevent the occurrence a greater proportion of cervical cancer and precancerous lesions were it to achieve wide- spread coverage (de Sanjosé *et al.*, 2011). Gardasil ™ (Merck &CO;Inc), Cervarix ™ (Glaxosmithkline) and Gardasil 9™ (Merk&Co;Inc) are the names of the three vaccines available for use against HPV infections (Herweijer et al., 2016; Joura et al., 2015). Each of these three vaccines fights against the acquisition of HPV 16 and HPV 18 infections which are known to cause over 70% of cervical cancer. The quadrivalent type has HPV 6 and HPV 11 added to fight against genital warts infection and finally the 9-valent vaccine has HPVs 31,33,45,52 and 58 added. Efficacy of these vaccines among HIV negative women is 98% for preventing type specific Cervical Intraepithelial Neoplasia (CIN) and 100% of all genital warts (Joura et al., 2015) Vaccine efficacy studies are needed on HIV positive women to establish the benefits of the vaccine to such a population. It has been recommended that HIV positive adolescents and adults be vaccinated between the ages of 13 to 25 (Masur et al., 2014). The right antibody response to the vaccine is needed to establish lifetime immunity among women living with HIV to prevent precancerous lesions and therefore cervical cancer. A study on sero-conversion among women

aged 13 to 45 showed highest conversion in those with CD4 count greater than 200 cells/µl compared to those lower than 200 cells/µl (Kojic et al., 2014). In another study, 90-100% sero-conversion rates were recorded in HIV infected boys and girls aged 7-12 years (Weinberg et al., 2012).

2.9.2 Universal/ Targeted Screening

The value of all cervical cancer screening techniques is in their ability to detect precursor cancer lesions in the cervix that can readily be remedied to avert cancer. Thus, screening is not a test for cancer. The paradigm of cervical cancer screening with use of cervical cytology (Pap smear) is the prime example of such a system. In advanced countries, strategic and universal cytology screening has massively reduced risk for cervical cancer. Regardless of the promise of universal vaccination, it is unlikely that screening programmes could be discontinued or even scaled-down for several reasons: immunization will only protect against HPV types that are targeted by the vaccine; protection will not be absolute and its longevity is uncertain; as yet, the possibility of genotype replacement cannot be excluded; and older women not covered by vaccination programmes will continue to be at risk.

2.9.3 Cervical Cancer Screening among HIV Positive Women

It is well known that, HPV infections are higher and can persist in women living with HIV and because of its persistence there is the need for regular screening to detect early any abnormality for prompt treatment to prevent the development of cancer of the cervix among this vulnerable group of women. It is recommended that such groups of women undergo Pap

test yearly for three years and if consecutively normal then the test is recommended every three years (Frellick, 2017).

The screening strategies being used are cytology based method with Papanicolaou test (Pap test), HPV DNA testing and visual inspection with acetic acid (VIA), visual inspection with Lugols iodine (VILI). In the less developed regions where there is limited resources and no instituted national cervical cancer control policy, a "see and treat" tactic is recommended by the World Health Organization (WHO). This provides an immediate treatment with cryotherapy without colposcopy and biopsy reducing loss to follow up rate. VIA has been found to show a good performance with a sensitivity of 48-76% for CIN2+ (Chibwesha et al., 2016; Firnhaber et al., 2013). Women living with HIV need exceptional precautions when using a VIA based screening model but testing for HPV provides a number of advantages which includes easy and a short time for sample collection compared to VIA and the likelihood of self-sampling.

2.10 SCREENING FOR CERVICAL DISEASE

2.10.1 Morphological Examination

Epithelial cell abnormalities usually develop without recognizable symptoms. Therefore the rationale for screening for cervical cancer has always been to detect abnormal cervical cells: including precancerous cervical lesions, as well as early cervical cancers at an early stage where remedial measures may be taken at reasonable cost. However, most women in countries with poorly-resourced healthcare systems do not have access to reliable screening programs

and consequently report to hospital only when lesions are at an advanced invasive stage. Women living with HIV are not excluded from this accessibility challenge. Overall screening and diagnosis is a major challenge in developing countries. The estimated coverage of cancer screening in developing countries is 19% compared to 63% in developed countries. However, in some areas, a screening rate of 1% or less has been observed in countries such as Bangladesh, Ethiopia, and Myanmar (Wabinga *et al.*, 2000; Clifford *et al.*, 2003).

2.10.2 DNA Testing for the presence of high-risk HPV types

HPV genomes and viral oncoproteins, which are present in all affected cells, represent convenient markers for a transient or persistent infection. The rationale for designing HPV DNA detection tests, also known as HPV testing, is based on the knowledge that persistent infection with high-risk HPV is a necessary cause of cervical cancer (Schiffman *et al.*, 1993; Walboomers *et al.*, 1999). The definitive diagnosis of current human papillomavirus (HPV) infection is made by detection of viral nucleic acids using molecular detection methods (Woodman *et al.*, 2001; de Sanjose *et al.*, 2003). Several methods of HPV testing have been developed to be able to do this effectively. In general, these tests can be grouped into (1) those reporting a pooled “high-risk” positive/negative result versus genotype specification, and (2) those targeting viral DNA versus viral mRNA or oncoprotein.

2.10.3 Indications for Cervical HPV DNA Testing

HPV DNA testing has also been recommended to replace or be combined with conventional cytological screening procedures but the former may be prohibitively expensive in settings where it is needed most. DNA testing is already employed as an adjunct to cervical cytology. Reflex HPV DNA testing is recommended in all cases of ASC-US. If high-risk HPVs are absent, the patient is returned to routine screening cervical cytology a year later. If high-risk HPVs are present, the patient is referred to immediate colposcopy with biopsy. If competently performed, these techniques clearly improve the detection rate of high-risk HPV DNA in women, and also avoid some false-negative diagnoses (Schlecht *et al.*, 2001).

In women who have had negative cytology but positive high-risk HPV DNA, repeat testing with both tests after a year may reassure the woman. Joint Pap and HPV testing is advocated to increase the sensitivity of either screening method for obstinate cervical lesions. According to some reports (Arbyn *et al.*, 2006a), a small percentage of patients testing positive for high-risk HPV types with normal cytology will go on to develop HSIL as early as six months on follow-up. Other unique scenarios supporting repeat HPV DNA testing are in the case of a persistent ASC-US Pap with negative colposcopic evaluation. Finally, those women that have had both negative cytology and negative high-risk HPV DNA on an annual screen may be candidates for combined testing every three years.

Chapter 3

MATERIALS AND METHODS

3.1 STUDY DESIGN AND SETTING

A cross-sectional observational study was undertaken among women living with HIV and attending clinic at the Korle-Bu Teaching Hospital. Women presenting for routine clinic were selected from May 2011 to December 2014 to participate in a study to characterize the cytological lesions and the prevalence of human papillomaviruses (HPV) as well as to screen for predictive factors for cervical abnormalities and high-risk infection among this population.

3.2 STUDY LOCATION

The study site was the HIV Clinic at the Korle-Bu Teaching Hospital (Latitude 5 degrees, 36 minutes north; Longitude 0 degrees, 10 minutes east). Korle-Bu Teaching Hospital is located at Guggisberg Avenue (10 minutes' drive under normal traffic from the central business centre of Accra, which is the capital town of the Greater Accra Region of Ghana. Accra has projected population of 4 million, making it the eleventh largest metropolis in Africa. Accra is sited in the coastal savannah ecological zone of Ghana with a land size of about 173 km^2. The average daily temperature is around 30^oC (86^oF) and receives an annual rainfall of between 600 mm and 800 mm.

Korle-bu Teaching Hospital was established on October 9, 1923 and it has grown from an initial 200 bed capacity to about 2,000. It is currently the third largest hospital in Africa and the leading national referral centre in Ghana (KBTH, 2017). It has currently 17 clinical and diagnostic departments/units and an average daily attendance of 1,500 patients with about 250 daily patient admissions. It has three centres of excellence, the National Cardiothoracic Centre, the National Reconstructive Plastic Surgery and Burns Centre and the National Radiotherapy

Centre (KBTH, 2017). In addition, it is the leading tertiary hospital in Ghana and serves as a research and training facility for students and researchers of the University of Ghana.

The HIV Clinic is located in the Fever's Unit under the Department of Medicine, KBTH. Currently the KBTH HIV Clinic has a 24-beds and runs the largest cohort of HIV patients in Ghana. Presently about 20,000 patients attend the clinic of which about 11,000 are on ART (KBTH, 2017). The Korle-Bu Teaching Hospital has been providing ART services to PLHIV since December 2003. The hospital runs the HIV Clinic three days in a week (Mondays, Wednesdays and Fridays) and operates an institutionalised electronic database which stores all clinic visits as well as medications, laboratory investigations and other patients' data. Currently the staff strength at the clinic is 5 clinicians, 30 nurses, 3 public health nurses, 8 pharmacists, 4 biomedical scientists, 6 volunteers, 8 counsellors and 5 data room staff. The fever's unit also houses cases of rabies and tetanus (3-bed capacity) (KBTH, 2017).

3.3 STUDY POPULATION AND SAMPLING

3.3.1 Study population

The study population is the 20,000 HIV-positive patients who attend the HIV clinic and the sampling frame is the electronic register of patients (KBTH, 2017). The patient population is made up of about 11,000 on ART with the rest on care. Each patient is scheduled to attend the clinic once every three months (at a minimum) for both clinical assessment and dispensing of medication (for patients on ARVs) (KBTH, 2017). Some patients have been on treatment for the past 11 years.

At HIV clinic enrolment, patients routinely present with baseline clinical and laboratory evaluations like physical examinations, full blood count, liver enzymes, serum creatinine, and

CD4+ T-cell count. Follow-ups are done every 3 months whilst occasional viral load is requested for when treatment failure is suspected since it is expensive and patients are unable to afford as a routine laboratory investigation. The recommended baseline CD4 + T-cell count for initiating ART was a count of 250 cells/μL or less and WHO clinical stage III/IV regardless of CD4+ count. This changed later to 350 cells/ μL or less in the 2011 guidelines and is presently pecked at 500 cells/ μL (NACP, 2014). Patients receive comprehensive care at a minimum cost of GH¢15 ($3.00) or receive free care when registered under the National Health Insurance Scheme. Irrespective of these requirements, patients not meeting the criteria are not excluded from receiving continuous care (KBTH, 2017). The care includes medical review, laboratory examinations, adherence counselling and general education on the disease and its management. Adherence is reviewed at every clinic visit and documented and is considered satisfactory when patients' self-report of compliance tallies with pill count (NACP, 2014).

3.3.2 Sampling Technique

A sample of 538 women attending HIV clinic at the Korle Bu Teaching Hospital were randomly selected to be part of the study. Any patient attending the clinic during the study period was given a chance to participate in the study after fulfilling the inclusion criteria.

Sampling was carried out on clinic days. List of clients attending clinic on each of these days was obtained and a sample size of 10/day was selected by computer generated random digit numbers. A maximum of 10 clients per clinic day was selected because of time constraint in taking the smears by the gynecologist within the clinic operating hours. Informed consent was

obtained from all women recruited for this study and clients were given the option of refusing participation without any effect on their treatment at the clinic.

3.3.3 Selection of study participants

A randomised selection of study participants was carried out as follows: The sampling unit was made up of individuals attending the HIV clinic which operates 3 days in a week (Mondays, Wednesdays and Fridays) and attends to about 150 patients on each of these days. Samples were picked from patients booked for each of the clinic days. Patients visit the clinic every three months which indicates that, a patient is likely to present to the clinic at least once every 36-clinic days. Sampling was done to give every patient an equal chance of being selected to be part of the study; hence, computer generated random sequence of eight unique code numbers was generated from the sampling frame for each clinic day for a 36 clinic day cycle (before the same group of patients returned for review). On each clinic day, five (5) patients were recruited into the study and sampled. Every patient in a particular day's list (sampling frame) was given a unique code number (extracted from folder number). Previously sampled patients were excluded from subsequent sampling frame based on date of last clinic attendance. After recruitment, patients were taken through the consent process and sampled only after satisfying the study inclusion/selection criteria. Recruited patients who did not satisfy the inclusion/selection criteria or who refused to take part in the study on any particular study day were not replaced but the enrolment procedure continued until the sample size estimated was reached. A total of 109 clinic days were used for the recruitment of study participants.

3.4 INCLUSION CRITERIA

All clients 18 years and above presenting to the clinic with a verifiable diagnosis of HIV infection were qualified to be included in the study. .

3.5 EXCLUSION CRITERIA

Individuals who failed to give consent were not allowed to participate in this study. Pregnant women and women who had undergone hysterectomy or conisation, or could not undergo an interview or a speculum vaginal examination were excluded from the study.

3.6 SAMPLE SIZE DETERMINATION

In the present study, a sample size of 538 women was considered ideal to afford adequate statistical power to achieve all the objectives and meet underlying statistical assumptions for inferential statistics. For objectives 1 and 4, to meet the objective of estimating the prevalence of a categorical dependent variable (presence of Pap smear abnormality) with two outcomes in a population of women attending clinic, a cohort of 334 individuals was calculated to give adequate power to the study. The minimum required sample size was calculated using StatCalc application of EpiInfo 3.5.3 using the formula $N= \underline{z^2\ p\ (1-p)}/d^2$ at 95% confidence interval. In this equation, N= desired sample size, z= the standard normal deviation (set at 1.96 corresponding to the 95% CI), p= the proportion in the target population estimated to have the outcome of interest, d= Precision or degree of accuracy desired (set at 0.05). In a previous study, the prevalence of Pap smear abnormalities among women attending reproductive health services was 44% (Dames *et al.*, 2009). For Objective 2, the aim was to estimate the occurrence of a categorical dependent variable (presence of high-risk HPV infection) with two outcomes

in the WLHIV. The prevalence of HR-HPV infection in the population was established at 23% (Donkoh, 2015). Similarly, after correcting for population size, it was determined that 270 subjects was a suitable sample size to estimate the prevalence of high-risk HPV infection among participants in this setting with precision (d=0.05) at 95% confidence level. For objectives 3 and 5, the minimum required sample size was calculated using the formula $N= \underline{z^2 p (1-p)}/d^2$ at 95% confidence interval. In this equation, N= desired sample size, z= the standard normal deviation (set at 1.96 corresponding to the 95% CI), p= the proportion in the target population estimated to have the outcome of interest, d= Precision or degree of accuracy desired (set at 0.05). In previous studies in sub-Saharan Africa, prevalence rates have ranged from 40-86% in women living with HIV (Yar *et al.*, 2015; Menon *et al.*, 2016). From this, a 50% prevalence of HPV was assumed yielding a maximum sample size estimate of 450. Taking all these sample requirements into consideration, five hundred and fifty women were selected in order to retain a dependable sample in the event of attrition and unforeseen participant absenteeism. Twelve patients were excluded for not giving consent and pre-analytical errors, leaving a final sample of 538 women.

3.7 Data Collection Tools: Pretesting and Administration

An in-depth, semi-structured, interviewer-administered questionnaire (Appendix 1) and all other data collection and screening tools were pre-tested for a week in a non-study area. Lessons learnt from the exercise provided a rational basis to restructure the format and presentation of interview guides and questionnaire for optimum achievement of study aims and benefits. The pre-tested questionnaire, which was in English, was administered in Twi by the investigator in the presence of the nurses as required. It was ensured that participants understood the questions first, before answering them. Information was sought on demographics and socioeconomic characteristics.

3.8 SPECMEN COLLECTION AND LABORATORY ANALYSIS/TESTS

3.8.1 Smear Preparation, Fixation and Transportation

Papanicolau (Pap) smear was done on all participants. Epithelial cells were obtained from the cervix with Pap Pak® cytology kit (Medical Packaging Corporation, Camarillo, CA, USA) using guideline provided by the producers of the product with minor alteration. Briefly, pre-labelled cervical smears were taken by performing vaginal speculum examination of the cervix, inserting the tip of the cervical spatula up through the cervical external os and rotating it around the ecto- and endo-cervix with special emphasis on the squamo-columnar junction. The sample on the spatula was rapidly but lightly stroked, thinly and evenly across the surface of the slide without any delay and immediately covered the slide with a mixture of 95% ethanol and 5% polyethylene glycol (carbowax) (BD-TriPath Imaging) according to the manufacturer's instructions. Endocervical smears were taken with brush applicator by rotating slightly in endocervical canal. Sampled cervical material was spread out evenly onto appropriate sections on slides by rolling endocervical brush across the glass slide from the frosted end towards the opposite end. The preparation was immediately fixed as before. Finally, slides were allowed to dry, packaged in mailing pouches and taken to the Cytology Laboratory accompanied with completed cytology request forms. The request forms accompanying the specimen were completed with the patient's name, a unique study and hospital code, age, date of collection of specimen, date of last menstrual period and other pertinent clinical information.

3.8.2 Staining and Screening of Cytological Slides

The modified Papanicolaou method that uses a standard nuclear stain, hematoxylin and two cytoplasmic counterstains (Orange-G-6 & Eosin Azure) has been recommended for the staining of cervical cytology slides (Koss and Melamed, 2006). Consequently, in order to

remove the carbowax protective layer, fixed smears were placed in 95% ethanol overnight. , Slides were then passed through a second alcohol wash to ensure complete removal of carbowax and hydrated under running tap water for 4 minutes. Slides were stained in Mayer's haematoxylin nuclear stain and blued in alkaline running water for 3 minutes each after arranging them in racks. Slides were then primed by washing them in 95% ethanol for 2 minutes after which they were stained in Orange G stain for 2 minutes and rinsed with 95% ethanol. Slides were then stained with Eosin Azure.

Finally, slides were washed in two changes of absolute ethanol to dehydrate them and cleared in xylene. DPX mountant that is compatible with the clearing agent, transparent and with a refractive index similar to the glass slides and specimen was used for binding the coverslip onto the slide (GCC Diagnostics, Gainland Chemical Co., Sandycroft Flints, UK). Slides were placed on the bench to air dry after which they were screened for any intra-epithelial lesions.

Slides were examined independently by two experienced cytotechnologists at the Korle-Bu Teaching Hospital. All positive cases, unsatisfactory smears and 10% random selection of all negative cases were reviewed by a senior pathologist for final interpretation, reporting and quality control.

3.8.3 Schedule for Repeat Cytology

Repeat sampling for all cases with unsatisfactory smears were performed after 8 weeks. This is because the scraped surface may not have re-epithelialized and the chance of a false negative result is increased before 6 weeks (Chan *et al.*, 2002).

3.8.4 DNA Sample Collection

Exfoliated cervical cells wre collected with the cytobrush after preparation of the Pap smears and washed into a pre-labelled tube containing DNA Guard (Biometrica, San Diego, USA), to preserve cellular DNA at room temperature (for up to three months) until DNA extraction using the QIAamp DNA Mini kit.

3.8.5 HPV-DNA Extraction, Detection and Genotyping

DNA was extracted using a commercial kit (Qiagen Ltd, Maryland, USA) using the manufacturer's instructions as shown in Appendix 2. Briefly, cervical cells were lysed by incubation at 56^{o}C with 20µl QIAGEN Proteinase K in a proprietary buffer AL (300 µl). Next, 400 µl ethanol (96–100%) was added to the extracted DNA to prime and bound to a special matrix in 2ml spin columns. The sample was washed with buffer AW1 and AW2 by centrifuging at 8000 rpm for 1 min and 14,000 rpm for 3 min respectively. The bound, pureDNA was eluted into 50µl Buffer AE and stored at -70^{o}C in duplicate until further processing.

HPV-DNA was detected and identified by nested multiplex PCR (NMPCR) (Sotlar *et al.*, 2004). A forward primer (GP-E6-3F) and two backward primers (GP-E7-5B and GP-E7-6B) constituted general primer PCR. The PCR reaction mix of 50µl contained 10X PCR buffer, 2.5 mM $MgCl_2$ 200µM of each of the four deoxyribonucleoside triphosphates (dNTP), 15pmols of each E6/E7 consensus primers and 1.25 units of Taq polymerase enzyme. Four microlitres (4µl) of DNA amplification reactions were carried out using extracts as templates. . This was done using a thermal cycler (BIOER GenePro thermocycler, BIOER Co., USA). The cycling parameters for the first round PCR with E63F/E75B/E76B consensus primers was 94^{o} C for four minutes, followed by 40 cycles of 94^{o} C for one minute, 40^{o} C for two minutes, 72^{o} C for two minutes and a single final elongation step of 72^{o} C for 10 minutes. In the second

round PCR, 2µl of first round PCR product, 15pmols of forward and reverse primers for genotyping were used. Primers for the identification of high-risk genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and low-risk genotypes 6/11, 42, 43, and 44 were used in four cocktails, each containing four to five different primer pairs (Appendix 3). For the second run, the parameters used in the first run PCR mix were kept but the cycling parameters were: : 94^{o} C for four minutes followed by 35 cycles of 94^{o} C for 30 seconds, 56^{o} C for 30 seconds, 72^{o} C for 45 seconds and a single final elongation step of 72^{o} C for four minutes (Sotlar *et al.*, 2004).

3.8.6 Analysis of Amplification Products

The amplified products were run by gel electrophoresis on 2% agarose gel and stained with 0.5µg/ml ethidium bromide. Ten microlitres of each sample was added to 2µl of orange G (5X) gel loading dye. The DNA marker used was a hundred base pair molecular weight (Sigma, MO, USA) which was run alongside the PCR products. The gel was prepared and run in 1X TAE buffer using a mini gel system at 100 volts for one hour and photograph taken over UV illuminator (Sotlar *et al.*, 2004).

3.9 DATA MANAGEMENT AND STATISTICAL ANALYSIS

The data obtained through respondents' questionnaires was checked for accuracy and entered immediately into the computer using Microsoft® Excel® (Microsoft Corporation, Redmond Campus, Washington DC, USA) microcomputer software. Data was analysed and managed using SYSTAT 12 for Windows (2007) (SYSTAT Software Inc., Florida, USA) and GraphPad Prizm 5.31 for Windows (2007) (GraphPad Software, San Diego California USA).

All HPV types were classified as low or high risk for high-grade cervical disease or as uncharacterized. HPV assignments were as described elsewhere (Munoz *et al.*, 2003a). HPV types grouped within the high-risk category included 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58,

59 and 68. HPV types grouped within the low-risk category included 6/11, 42, 43, 44 and 66. Women with multiple HPV types were considered to be in each risk category on the basis of the detection of any 1 of the high-risk or low-risk, HPV types. Analyses were not restricted to women with only single HPV infections.

A goal of the statistical analysis was to describe the relationship of sex history and age to the detection of high-risk, low-risk, and uncharacterized HPV types, as well as to evaluate additional subject characteristics for their association with the detection of these HPV groups after controlling for sex history and age. Sex history included number of lifetime sex partners, number of sex partners in the past year, age at first intercourse, number of years since first sexual intercourse, and sex history of the current sex partner. Additional subject characteristics included age, ethnicity, marital status, education level, age at menarche, parity, smoking history, history of use of condoms, and birth control pills. Defining the relationship among HPV detection, sex history, and age was done with simple contingency tables.

Initial analysis was done to acquire descriptive statistics. Categorical variables were compared using Chi square test and student t-test was used for quantitative variables. For hypotheses comparing frequencies among groups, X^2 tests was performed; comparisons of subgroups (e.g., gender, race) was assessed by logistic regression. The multivariate binary logistic regression model was used to determine the odds of the predictors on the dependent variable. A backward stepwise regression selection was adopted for the outcome and the predictor variables. Several models were fitted.

For Objective 4, the question to be addressed was, which of the explanatory variables are predictive of abnormal cytology? Investigation of the determinants of abnormal cytology proceeded in two steps. First, unadjusted relationships between abnormal cytology and potential predictor variables were examined singly with margin of error set at .30. Then, the regression

procedure was repeated to create the best model for predicting the effect of potential variables on cytology outcome. In the multivariate logistic regression model, age, educational status, occupation, marital status, income, ethnicity, age at first pregnancy and age at sexual debut were initially entered in one step. Then non-significant variables were removed in stepwise fashion to give the best fit model. The final model based on educational status, income, and age at sexual debut. These steps were repeated for behavioural and clinical characteristics of women. In the initial model, smoking history, current tobacco use, condom use, HIV duration, ART use, ART duration, discharge, sores and CD4 count were loaded in one step. The final model was based on ART use and CD4 count.

For Objective 5, the question to be addressed was, which of the explanatory variables were predictive of high-risk HPV infection? Investigation of the determinants of high-risk HPV infection proceeded in two steps. First, unadjusted relationships between high-risk HPV infection and potential predictor variables were examined singly with margin of error set at .30. In univariate analysis, formal education and estimated income had significant main effects for high-risk HPV infection and therefore were associated with high-risk infection. This relationship did not disappear after adjusting for potential confounders such as woman's age, younger age at sexual debut and recent sexual activity. Recent sexual activity is defined for a woman who reported having a sexual partner in past six months. The final regression model was based on age, educational status, occupation, marital status, income, ethnicity, age at first pregnancy and age at sexual debut.

3.10 ETHICS APPROVAL

Approval was sought and obtained from the Korle Bu Teaching Hospital Ethical Committee and the College of Health Sciences ethics review board (MS-Et/M-P4.6/2012-2013). Study participants were sufficiently told of the reasons, nature, processes, risks and dangers of the

procedure. Importance was placed on anonymity, secrecy and the free will to decide not to be part of the study any time without any consequences.

Informed consent: All study subjects consented by signatures.t. Participants were fully informed about the purpose, procedures, risks, and benefits of participating in this study. Those who agreed to participate signed or thumb printed an informed consent sheet.

Confidentiality: Each participant's responses were kept confidential and data collected were kept for the purpose of this study only.

Safety procedures: Cytology samples were taken strictly by gynaecologists who have been taking samples for more than one year. Women showing signs of sexually transmitted infection e.g. (cervicovaginal discharge) were referred for treatment and asked to return to the clinic after two weeks for study screening and enrolment.

Chapter 4
RESULTS

4.1 Demographic Profile of Study Population

The presentation of the demographic and behavioral profile of the population is important because this study investigated the association of some demographic and behavioural characteristics with the detection of 18 low-risk and high-risk genital HPVs in HIV-infected women recruited at the Korle-Bu Teaching Hospital for cervical screening. Cervical samples were obtained from 538 women aged 20 to 69 years. The mean age of the participants was 40.7 years (SD=8.4years). Age and all other continuous variables studied were found to follow a Gaussian distribution according to D'Agostino-Pearson normality test.

Percentages were calculated based on the total number of respondents to each demographic parameter. The four major ethnic groupings in Ghana were represented in the study as follows: Akan (48.8%), Mole-Dagbani (16.9%), Ewe (19.5%) and Ga (19.3%). Illiteracy was reported by 23.8% of the women. The greater fraction of the study population had completed primary school (57.4%), while 4.6% had accessed tertiary level education. Married women formed 38.3% and 23.4% were widowed. Single women accounted for less proportion of 2.0% of the study participants. With regards to employment, majority of the women were involved in non-formal work, mostly petty trading, and accounting for 78.8%.

Table 4.1-1: Socio-demographic characteristics of Women Living with HIV (WLHIV) at the Korle Bu Teaching Hospital, Accra,

Variable	Frequency	Percentage	Valid responses
Age (years)			**538**
<25	6	1.1	
25-34	118	21.9	
35-44	248	46.1	
45-54	137	25.5	
55-64	29	5.4	
Formal education			**538**
None	128	23.8	
Primary	309	57.4	
Secondary	76	14.1	
Tertiary	25	4.7	
Occupation			**538**
Civil servant	35	6.5	
Unemployed	79	14.7	
Non-formal work	424	78.8	
Marital status			**5**
Divorced	96	17.8	
Married	206	38.3	
Single	110	20.5	
Widowed	126	23.4	
Income level (Gh¢/month)			**532**
<100	138	26.0	
100-249	254	47.7	
250-500	41	7.7	
>500	27	5.1	
Unsure	72	13.5	
Ethnicity			**532**
Akan	230	43.2	
Ewe	105	19.7	
Ga	104	19.5	
Mole-Dagbani	91	17.1	
Others	2	0.4	

Table 4.1-2: Reproductive characteristics of WLHIV at the Korle Bu Teaching Hospital, Accra,

Variable	Frequency	Percentage	Valid responses
Only wife			**507**
Yes	494	97.4	
No	13	2.6	
Other wives			**13**
1	11	84.6	
2	2	15.4	
Recent sexual partners			**538**
Yes	265	49.3	
No	273	50.7	
Lifetime sexual partners			**263**
<3	63	24.0	
3-6	185	70.3	
>6	15	5.7	
Age at coitache			**531**
<16	81	15.2	
16-18	247	46.5	
>18	203	38.2	
Age at first pregnancy			**511**
<18	91	17.8	
18-25	333	65.2	
>25	87	17.0	
Number of pregnancies			**511**
<2	73	14.3	
2-4	252	49.3	
>4	186	36.4	
Number of miscarriages			**511**
None	386	75.5	
1	73	14.3	
2	29	5.7	
>2	23	4.5	
Induced abortions			**511**
none	255	50.0	
1	96	18.8	
2	72	14.1	
>2	88	17.2	
Number of still-births			**511**
None	484	94.7	
1	24	4.5	

2	3	0.6	
Condom use			**538**
Never	256	47.6	
Sometimes	137	25.5	
Always	145	27.0	

Table 4.1-3: Clinical characteristics of WLHIVat the Korle- Bu Teaching Hospital, Accra,

Variable	Frequency	Percentage	Valid responses
HIV duration (months)			**535**
<48	118	22.1	
48-71	119	22.2	
72-95	104	19.4	
96-119	106	19.8	
>119	88	16.4	
ART use			**511**
Yes	429	84.0	
No	82	16.o	
ART duration (months)			**424**
<30	148	35.0	
30-59	95	22.4	
60-89	128	30.2	
>89	53	12.5	
Vaginal discharge			**537**
Yes	35	6.5	
No	502	93.5	
Presence of sores			**537**
Yes	10	1.9	
No	527	98.1	

The proportion of women involved in polygamous marriages were 2.4% and half of them (50.7%) had not been sexually active within the last six months prior to the time of administering the questionnaire. For those who responded to the question on "lifetime sexual partners" 34% had between three to six partners and 2.8% had above six partners. Approximately half of the women (45.9%) had their first sexual encounter between the ages of 16 and 18 years whiles 15.1% had theirs below 16 years of age. A greater proportion of the women (61.9%) had their first conception between the ages of 18 and 25 years and 46.8% had between 2 and 4 conceptions but the average number of lifetime pregnancies was 4.10 (SD=2.71) and a few reported from 10 to 32 pregnancies. Majority (71.7%) had no miscarriages and 47.4% had not had any abortions. Almost all the women (90.0%) had never had a still birth and approximately half had never used condoms before whiles 27.0% always use condoms. Women on ART accounted for 79.9%, there was no vaginal discharge in 93.3% and no vaginal sore (98.0%).

4.2 Proportion of WLHIV with Cervical Cytology Abnormalities

Out of the 538 women for whom a Pap smear was available for evaluation, 433 (80.5%) were negative for intraepithelial lesion or malignancy. In addition, 26 slides were unsatisfactory for evaluation due to various reasons and 27 slides had neither transformation zone components nor features of dyskaryosis/intraepithelial lesions (Table 4.2-1). Although these were considered negative, false negatives are likely to be included since sampling of the transformation zone is not assured. In all, the proportion of women with abnormal cytology was 9.7% (52 out of 538 women). Fifteen women (2.8%) showed atypical squamous cells of undetermined significance (ASCUS), 21 (3.9%) low-grade squamous intraepithelial lesions, and 15 (2.8%) high-grade squamous intraepithelial lesions. None of the women was

suspected to have invasive squamous cell carcinoma and none was found to have atypical glandular cells or adenocarcinoma. Other infections were detected. Among all women screened, 69 (12.8%) had bacterial vaginosis alone, 30 (5.6%) had vaginitis alone, 14 (2.6%) had candidiasis alone and 1 (0.2%) had *Trichomonas vaginalis* infection. Four representing 0.7% of the women had bacterial vaginosis and *Trichomonas vaginalis* together.

Table 4.2-1: Summary of Papanicolau Test Results for WLHIV at the Korle Bu Teaching Hospital, Ghana,

Cytology Result according to Bethesda System		
	Frequency (N)	**Percentage (%)**
Abnormal cervical cytology		
ASCUS	15	2.8
ASC-H	1	0.2
LGSIL	21	3.9
HGSIL	15	2.8
Normal cervical cytology		
NILM*	433	80.5
No TZC	27	5.0
Unsatisfactory	26	4.8
Total	538	100.0

NILM: Negative for intraepithelial lesions or malignancy, ASCUS: Atypical squamous cells of unknown significance, ASC-H: Atypical squamous cells, cannot rule out HSIL, LGSIL: Low-grade squamous intraepithelial lesions, HGSIL: high-grade squamous intraepithelial lesions.

4.3 Prevalence of HPV Genotypes among Unscreened Women Living with HIV

Cervical swabs were obtained from 538 HIV-positive women with no history of Pap smear screening and analysed for 18 HPV types. Table 4.3-2a shows the prevalence of HPV types detected in smears from these women. The prevalence of low-risk HPV (LR-HPV), types 6/11, 42, 43, 44 and 66, is shown in Table 4.3-2b and that of high-risk HPV (HR-HPV), types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 in Table 4.3-2c. The occurrence of HPV DNA was 52.0% (280/538 women, 95% CI: 47.7-56.3). The prevalence of LR-HPV without taking note of other types was 22.3% (120/538 women; 95% CI 18.8-26.1) while that of HR HPV without accounting for other types was 41.8% (225/538 women; 95% CI 37.6-46.1). LR HPV only infection was seen in 10.2% (55/538 women, 95%CI 7.8-13.1). HR HPV only infection was seen in 29.7% (160/538 women, 95% CI 25.9-33.8). Both HR and LR were seen in 12.1% (65/538 women, 95%CI 9.4-15.1).

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Table-4.3-1: Prevalence of human papillomavirus (HPV) in WLHIV at the Korle Bu Teaching Hospital, Accra, Ghana,.

Parameter		**Frequency**	**Percentage**
HPV present (**N=538**)			%[a]
	Yes	280	52.0
	No	258	48.0
Type of HPV detected (**N=280**)			%[b]
	HR HPV only	160	57.1
	LR HPV only	55	19.6
	Both HR & LR HPV	65	23.2
Number of HPV genotypes detected (**N=280**)			%[b]
	Single	151	53.9
	Multiple	129	46.1

[a] Percentages calculated using total number of cases (N= 538) as common denominator.
[b] Percentages calculated using number of positive cases (N= 280) as common denominator.
[α] Women infected with only LR HPV. Low-risk HPV types include 6/11, 42, 43, 44 and 66.
[β] Women infected with only HR HPV. High-risk HPV types include 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68.

All 18 HPV genotypes screened were detected. The most common HR-HPV types were (Table 4.3-2), HPV-35 with 14.1% (76 out of 538 women, 95%CI 11.3-17.4), HPV-18 (13.0%; 70 out of 538 women, 95%CI 10.3-16.1), HPV-58 (7.4%; 40 out of 538 women, 95% CI 5.4-10.0), HPV-52 (7.2%; 39 out of 538 women), and HPV-45 (4.8%; 26 out of 538 women, 95% CI 3.2-7.0). HPV 42 with 7.8% (39 out of 538 women, 95% CI 5.2-9.8) was the commonest LR-HPV type detected (Table 4.2-3). The frequency of HPV 16 (the main HR-HPV type linked with cervical cancer worldwide) was 2.4% (13 out of 538 women, 95%CI 1.3-4.1).

Table 4.3-2: Prevalence of high-risk HPV Genotypes in WLHIV at the Korle Bu Teaching Hospital, Accra, Ghana,

HPV type	n*	%[a]	%[b]	%[c]
HPV-52	39	7.2	14.1	17.3
HPV-56	9	1.7	3.2	4.0
HPV-35	76	14.1	27.4	33.8
HPV-18	70	13.0	25.3	31.1
HPV-58	40	7.4	14.4	17.8
HPV-68	16	3.2	5.8	7.1
HPV-51	12	2.2	4.3	5.3
HPV-39	18	3.3	6.5	8.0
HPV-45	26	4.8	9.3	11.6
HPV-16	13	2.4	4.7	5.8
HPV-59	7	1.3	2.5	3.1
HPV-33	5	0.9	1.8	2.2
HPV-31	9	1.7	1.1	4.0
HPV-44	20	3.7	7.2	8.9
HPV-66	3	0.6	1.1	1.3

* Because of multiple infections, women may be counted more than once.
[a] Percentages calculated using total number of cases (N= 538) as common denominator.
[b] Percentages calculated using number of positive cases (N= 277) as common denominator
[c] Percentages calculated using number of HR cases (N= 225) as common denominator.

Table 4.3-3: Prevalence of low-risk HPV Genotypes in WLHIV at the Korle Bu Teaching Hospital, Accra, Ghana,

HPV type	n*	%[a]	%[b]	%[c]
HPV-42	45	8.4	16.2	37.8
HPV-43	28	5.2	10.1	23.5
HPV-66	3	0.6	1.1	2.5
HPV-6/11	32	5.9	11.6	26.9
HPV-44	20	3.7	7.2	16.8

* Because of multiple infections, women may be counted more than once.
[a] Percentages calculated using total number of cases (N= 538) as common denominator.
[b] Percentages calculated using number of positive cases (N= 277) as common denominator
[c] Percentages calculated using number of LR cases (N= 119) as common denominator.

Study population demographics and human papillomavirus (HPV) genotype prevalence estimates are presented in Table 4.3-4 for all infected women. Row percentages are computed to show within-group HPV prevalence estimates. The estimated age-related prevalence rates ranged from 0.7% in women aged below 25 years to 49.7% in women between the ages of 35 and 44 years. Women who had primary level education had low-risk HPV prevalence of 35.2% compared to 63.6% in those with tertiary level education. The prevalence of high-risk HPV was also 63.6% in those with tertiary level and 87% in women with primary level education. The prevalence of HPV across the different ethnic groups was approximately the same however, the Mole-Dagbani had the highest prevalence of 56.0%.

Table 4.3-4: Study population demographics and HPV prevalence in WLHIV at the Korle-Bu Teaching Hospital, Accra, Ghana,

		Prevalence of HPV, %[#]		
Characteristic	**No. (%*)**	**Low-risk HPV**	**High-risk HPV**	**Any HPV**
Age ***(N=532)***				
25-34	118 (22.2)	40.4	77.2	46.6
35-44	248 (46.6)	44.0	80.6	54.0
45-54	137 (25.8)	39.4	81.7	51.1
55-64	29 (5.5)	50.0	81.2	55.2
Education ***(N=538)***				
Primary	309 (57.4)	35.2	87.0	51.8
Secondary	76 (14.1)	50.0	72.7	56.6
Tertiary	25 (4.6)	63.6	63.6	44.0
None	128 (23.8)	52.4	71.4	49.2
Marital status ***(N=538)***				
Divorced	96(17.8)	41.7	77.1	49.0
Married	206 (38.3)	36.5	82.3	46.1
Single	110 (20.4)	51.7	75.9	52.7
Widowed	126 (23.4)	53.8	83.3	61.1
Ethnicity ***(N=530)***				
Akan	230 (43.4)	38.2	85.5	47.0
Ewe	105 (19.8)	48.2	76.8	52.4
Ga	104 (19.6)	37.5	78.6	53.8
Mole-Dagdani	91 (17.2)	47.2	74.5	56.0
Parity				
Gravidae (N=511)				
Less than 2	73 (14.3)	46.2	76.9	53.4
2 to 4	252 (49.3)	46.5	75.6	50.0
Greater 4	186 (36.4)	36.4	86.9	52.2

[#] Row percentages are computed to show within-group HPV prevalence estimates.
*****Percentage fraction of respondents**.**

Table 4.3-4 continued

Characteristic	No. (%*)	Prevalence of HPV, %[#]		
		Low-risk HPV	High-risk HPV	Any HPV
Abortion (N=256)				
1	96 (37.5)	47.6	76.2	43.8
2	72 (28.1)	43.2	84.1	61.1
>2	88 (34.4)	40.4	84.6	56.8
Miscarriage (N=125)				
1	73 (58.4)	42.5	82.5	53.4
2	29 (23.2)	22.2	88.9	31.0
>2	23 (18.4)	-	100.0	43.5
Age at first pregnancy (years) ***(N=511)***				
≤17	91 (17.8)	44.2	79.1	47.3
18-25	333 (65.2)	41.0	80.9	52.6
>25	87 (17.0)	47.7	77.3	50.6

[#] Row percentages are computed to show within-group HPV prevalence estimates.
*****Percentage fraction of respondents**.**

Table 4.3-5: Sexual history and HPV prevalence in WLHIV at the Korle-Bu Teaching Hospital, Accra, Ghana,

		Prevalence of HPV, %[#]		
Characteristic	**No. (%)**	**Low-risk HPV**	**High-risk HPV**	**Any HPV**
Age at maiden sexual encounter *(N=531)*				
< 16	81 (15.2)	42.9	76.2	51.9
16-18	247 (46.5)	41.1	82.3	49.0
>18	203 (38.2)	43.5	79.6	53.2
HIV duration (months)[a] *(N=531)*				
<48	118 (22.1)	32.4	81.1	*61.9*
48-71	119 (22.2)	51.0	78.4	*41.2*
72-95	104 (19.4)	46.3	75.9	51.9
96-119	106 (19.8)	45.6	82.5	53.8
>119	88 (16.4)	42.9	83.3	47.7
lifetime sex partners *(N=263)*				
<3	63(24.0)	50.0	80.0	63.5
3-6	185 (70.3)	43.4	78.7	64.9
>6	15 (5.7)	50.0	87.5	53.3
ART*(N=511)*				
Yes	429 (83.9)	43.4	80.3	52.7
No	82 (16.1)	27.8	91.7	42.7
ART duration (months) *(N=424)*				
<30	148 (34.9)	39.7	84.6	52.0
30-59	95(22.4)	49.1	67.9	54.7
60-89	128(30.2)	48.3	81.7	46.9
>89	53(12.5)	39.4	84.8	62.3
Wife of monogamous relationship [a]*(N=269)*				
No	117 (43.5)	44.4	77.8	69.2
Yes	152 (56.5)	35.3	82.4	43.1

[#] Row percentages are computed to show within-group HPV prevalence estimates.
*****Percentage fraction of respondents**.**
[a] Prevalence of HPV (any type) varies significantly across categories.

Characteristic	No. (%*)	Prevalence of HPV, %[#]		
		Low-risk HPV	High-risk HPV	Any HPV
Condom use	***(N=538)***			
Always	145 (27.0)	49.3	73.1	44.8
Never	256 (49.2)	36.4	86.0	50.0
Sometimes	137 (25.5)	46.4	77.4	61.8
Ever smoked tobacco	***(N=536)***			
Yes	6 (1.1)	25.0	100.0	66.7
No	530 (98.9)	42.9	80.0	51.3
Vaginal Discharge	***(N=537)***			
Yes	35 (6.5)	40.0	84.0	68.6
No	502 (93.5)	42.9	79.9	50.2
Skin Ulcers*(N=537)*				
Yes	10 (1.7)	75.0	87.5	80.0
No	527 (98.1)	41.7	80.1	50.9

[#] Row percentages are computed to show within-group HPV prevalence estimates.
*****Percentage fraction of respondents**.**
[a] Prevalence of HPV (any type) varies significantly across categories.

Tables 4.3-6, 4.3-7 and 4.3-8 shows HPV type distribution in women living with HIV at the Korle-Bu Teaching Hospital, Accra, stratified according to cytological outcome (presence or absence of cervical cytology abnormality) and number of detected HPV genotypes. The number of women with an LR-HPV genotype infection (irrespective of the presence of other HPV types) was calculated using 538 as the denominator and separated under two categories: single and multiple infections. The results are presented based on the presence or absence of cervical cytological dysplasia. There were 26 unsatisfactory slides out of which17 had HPV infection: 7 had multiple HPV infections and 10 had single HPV infections (the distribution of HR- and LR-HPV in rejected slides are given in Table 4.3-9).

The majority of women 86.2% (464/538 women, 95% CI 83.0-89.0 :) had normal cervical findings. Only 9.7% (52/538 women, 95% CI 7.3-12.5 (9.7%) had a concurrent abnormal Pap smear result. Among women who had abnormal cervical results, the prevalence of HPV infection was 50.0% (26/52 women, 95% CI 35.8-64.2) compared to 51.5% (239/464 women, 95%CI 46.8-56.1) who had normal cytology results. Women who had abnormal Pap smear findings did not have a disproportionate prevalence of HR-HPV infection (χ^2=0.01, df=1, P=0.91). The prevalence of HR HPV infection was 80.8% among women with abnormal simultaneous pap result compared to 79.9% among women with normal Pap smear findings. Again, women with atypical pap smears did not show a greater tendency to harbour multiple HR-HPV infections compared to women with normal pap smears (χ^2=0.57, df=1, P=0.45). Multiple infections were more likely to be associated with HR-HPV genotypes than with LR-HPV infection (χ^2=122.3, df=2, P=0.00).

Table 4.3-6: HPV type distribution stratified according to concurrent cytology outcome and number of detected HPV genotypes HPV Prevalence and cytological outcomes in WLHIV at the Korle Bu Teaching Hospital

	Pap test								
	Normal			Abnormal			Total		
HPV type	**Single**	**Multiple**	**count (%[a,b])**	**Single**	**Multiple**	**count (%[c,d])**	**Single**	**Multiple**	**Total (%)**
			n= 464 (86.2)			***n= 52 (9.7)***			***n= 538***
HPV negative			225 (48.5[a])			26 (50.0[c])			251 (46.7[e])
HPV positive	129	110	239 (51.5[a])	12	14	26 (50.0[c])	141	124	265 (49.3[e])
HR HPV positive [α]	81	110	191 (79.9[b])	8	13	21 (80.8[d])	89	123	212 (75.7[f])
LR HPV positive [β]	48	54	102 (42.7[b])	4	9	13 (50.0[d])	52	63	115 (41.1[f])

[α] All women harbouring at least one HR HPV infection. Cases with more than one HR infection are counted once.
[β] All women harbouring at least one LR HPV infection. Cases with more than one LR infection are counted once.
%[a]: out of 464 women with normal cytology
%[b]: out of 239 HPV positive cases with normal cytology
%[c]: out of 52 women with abnormal cytology
%[d]: out of 26 HPV positive cases with abnormal cytology
%[e]: out of 538 WLHIV
%[f]: out of 280 HPV positive WLHIV, not excluding 22 unsatisfactory pap smears absent from table 4.4-1

Table 4.3-7: Prevalence of LR HPV types by cytological diagnosis at the Korle-bu Teaching Hospital,Accra, Ghana,.

	Pap test								
	Normal			Abnormal			Total		
HPV type	**Single**	**Multiple**	**Total (%[#])**	**Single**	**Multiple**	**Total (%[#])**	**Single**	**Multiple**	**Total (%[#])**
Low-risk infections									
42 [a]	17	16	33 (73.3)	3	5	8 (17.8)	20	21	41 (91.1)
43	8	17	25 (89.3)	0	3	3 (10.7)	8	20	28 (100.0)
66	0	2	2 (66.4)	0	1	1 (33.3)	0	3	3 (100.0)
44	13	5	18 (90.0)	1	1	2 (10.0)	14	6	20 (100.0)
6/11	8	20	28 (87.5)	1	2	3 (9.4)	9	22	31 (96.9)
sub-total	**46**	**60**	**106 (82.8)**	**5**	**12**	**17 (13.3)**	**51**	**72**	**123 (96.1)**

[#] Row percentages are computed to show within-group HPV prevalence estimates. May not add up to 100% because of 22 unsatisfactory cytological slides

[a] Prevalence of HPV (any type) varies significantly across categories.

Table 4.3-8: Prevalence of HR HPV types by cytological diagnosis at the Korle-Bu Teaching Hospital, Accra, Ghana,

	Pap test								
	Normal			Abnormal			Total		
	Single	Multiple	Total (%[#])	Single	Multiple	Total (%[#])	Single	Multiple	Total (%[#])
High-risk infections									
52	9	24	33 (84.6)	0	4	4 (10.3)	9	28	37 (94.9)
35	20	44	64 (84.2)	3	6	9 (11.8)	23	50	73 (96.1)
18	11	49	60 (85.7)	1	5	6 (8.6)	12	54	66 (94.3)
58	8	24	32 (80.0)	0	5	5 (12.5)	8	29	37 (92.5)
56	4	4	8 (88.9)	0	1	1 (11.1)	4	5	9 (100.0)
39	8	7	15 (83.3)	1	2	3 (16.7)	9	10	19 (100.0)
51	3	6	9 (75.0)	0	2	2 (16.7)	3	8	11 (91.7)
59	1	5	6 (85.7)	0	0	0 (0.0)	1	5	6 (85.7)
45	6	16	22 (84.6)	0	4	4 (15.4)	6	20	26 (100.0)
16	2	9	11 (84.6)	0	2	2 (15.4)	2	11	13 (100.0)
68	7	20	27 (81.8)	1	2	3 (9.1)	8	22	30 (90.9)
33	1	4	5 (100.0)	0	0	0 (0.0)	1	4	5 (100.0)
31	2	6	8 (88.9)	1	0	1 (11.1)	3	6	9 (100.0)
sub-total	**82**	**218**	**300**	**7**	**33**	**40**	**89**	**252**	**341 (95.7)**

[#] Row percentages are computed to show within-group HPV prevalence estimates. May not add up to 100% because of unsatisfactory cytology slides

[a] Prevalence of HPV (any type) varies significantly across categories.

The present study investigated the relationship between detection of HPV genotypes and the presence of specific diagnostic cytological abnormality in women living with HIV at the Korle-Bu teaching hospital, Accra. Table 4.3-9 presents the distribution of all types of HPV, low risk and high-risk HPVs and the corresponding diagnostic category for these women. Prevalence of HPV infection ranged from 25.9% (95% CI 11.1-46.3) among women with no TZC to 56.3% (95% CI32.3-83.7) among women with ASC and 65.4% (95% CI 44.3-82.8) in women with unsatisfactory smears. The cross-tabulation procedure in SPSS helps to evaluate differences in distribution of various cytology outcomes and the prevalence of low risk, high-risk and any type HPV among these women. In this regard, no significant difference was noted for the any type HPV infection, high-risk HPV, or low-risk HPV and Pap smear outcome (Table 4.3-9).

Table 4.3-9: Distribution of low risk, high risk and any type HPV in concurrent cervical cytology outcomes for 538 WLHIV screened at Korle Bu Teaching Hospital, Accra,

Characteristic	No. (%[#])	Prevalence of HPV, %*		
		Any HPV[a]	High-risk HPV	Low-risk HPV
No TZC	27 (5.0)	7 (25.9)	5 (18.5)	2 (7.4)
NILM	433 (80.5)	230 (53.1)	184 (42.5)	99 (22.9)
ASCUS	15 (2.8)	9 (60.0)	9 (60.0)	2 (13.3)
LSIL	21 (3.9)	9 (42.9)	6 (28.6)	7 (33.3)
ASC-H	1 (0.2)	1 (100.0)	1 (100.0)	0 (0.0)
HSIL	15 (2.8)	7 (46.7)	5 (33.3)	4 (26.7)
Unsatisfactory	26 (4.8)	17 (65.4)	15 (57.7)	6 (23.1)
(χ^2, df=5, P-value)		*10.2; 0.12*	*4.91; 0.56*	*8.36; 0.21*

[#] Percentage fraction of respondents**.**
***** Row percentages are computed to show within-group HPV prevalence estimates.
[a] Prevalence of HPV (any type) varies significantly across categories (Pearson x^2 test).

Table 4.3-10 shows the exclusive distribution of HPV genotypes in the 538 women according to concurrent cytological diagnosis. Each genotype found in multiple infection was treated as an isolated event and tallied exclusively. The commonest HPV genotypes detected among women with ASCUS were HPV-18 (20% (95%CI 4.3-48.1) of all ASCUS cases), 35 (20% (95%CI 4.3-48.1) of all ASCUS cases) and 31, 39, 45, 51, 52, 58, 68 (6.7% (95%CI 0.2-31.9) of all ASCUS cases each). Among women with LSIL most prevalent were HPV-52 (28.6% (95%CI 11.3-52.2) % of all LSIL cases), HPV-35 (14.3% (95%CI 3.0-36.3) of all LSIL cases), and HPV 16,18, 58,39 (9.5% (95%CI 1.2-30.4) of all LSIL cases). HPV-52, 35 (20% (95%CI 4.3-48.9) of all HSIL cases) and 58,45 (13.3% (95%CI 1.7-40.5) of all HSIL cases) were top three in that order for high grade intraepithelial/severe dysplasia lesions.

HPV-35 (14.8% (95%CI 11.6-18.5) of all normal cases), HPV-18 (13.2% (95%CI 10.1-16.7) of all normal cases), HPV-52 (7.6% (95%CI 5.3-10.5) of all normal cases), HPV-58 (7.2% (95%CI 4.9-10.0) of all normal cases) and HPV-68 (5.8% (95%CI 3.8-8.4) of all normal cases) was more commonly detected in women without any precancerous lesions.

Table 4.3-10: Distribution of HPV genotypes among 538 WLHIV screened at Korle Bu Teaching Hospital, Accra,

HPV genotype	ASCUS	LSIL	HSIL	ASC-H	NILM
	N=15 (%)	*N=21 (%)*	*N=15 (%)*	*N= 1 (%)*	*N=433 (%)*
High-risk infections					
52	1(6.7)	6 (28.6)	3(20.0)	-	33(7.6)
35	3(20.0)	3(14.3)	3(20.0)	-	64(14.8)
18	3(20.0)	2 (9.5)	1(6.7)		57(13.2)
58	1(6.7)	2 (9.5)	2(13.3)	-	31(7.2)
56	-	-	1(6.7)	-	7(1.6)
39	1(6.7)	2 (9.5)	-	-	14(3.2)
51	1(6.7)	-	1(6.7)	-	9(2.1)
59	-	-	-	-	6(1.4)
45	1(6.7)	1(4.8)	2(13.3)	1(100.0)	20(4.6)
16		2 (9.5)	-	-	10(4.0)
68	1(6.7)	1(4.8)	1(6.7)	-	25(5.8)
33	-	-	-	-	5(1.2)
31	1(6.7)	-	-	-	7(1.6)
Low-risk infections					
42	2(13.3)	3(14.3)	3(20.0)	-	33(7.6)
43	-	2 (9.5)	1(6.7)	-	25(5.8)
66	1(6.7)	-	-	-	2(0.5)
44	1(6.7)	1(4.8)	-	-	17(3.9)
6/11	1(6.7)	2(9.5)	-	-	26(6.0;)

4.4 Correlates of abnormal cytology and their implications for disease prevention

Tables 4.6-1 and 4.6-2 show the crude and adjusted odds ratios (ORs) for Pap smear abnormality and corresponding 95% confidence intervals (CIs) according to socio-demographic and reproductive characteristics among women. However, after adjusting for potential confounders such as woman's age and age at first pregnancy, younger age at sexual debut had significant main effect for abnormal Pap smear result later in the life of women in the study. Then, the regression procedure was repeated to create the best model for predicting the effect of potential variables on cytology outcome. The final model- shown here in Table 4.6-3 was based on educational status, income, and age at sexual debut, significantly predicted Pap smear results (x^2 =28.816, df= 8, N=504, p=0.002) and explained up to 10% (Nagelkerke R^2=0.096) of the variability in and cytology outcome.

Table 4.4-1: Odds ratios (ORs) for cervical dysplasia and matching 95% confidence intervals (CIs) according to socio-demographic characteristics in WLHIV at Korle Bu Teaching Hospital, Accra,

Parameter	Pap smear abnormality								
	N(%)	*#n(%)*	*χ^2 (p-value)*	*β (p-value)*	*OR*	*95% CI*	*µβ (p-value)*	*µOR*	*95% CI*
Age*			13.2 (0.11)	0.29			-0.33 (0.143)	0.967	0.93-1.01
<25	6 (1.1)	2 (33.3)			1				
25-34	118 (21.9)	16 (13.6)		-1.13 (0.21)	0.32	0.06-1.91			
35-44	248 (46.1)	21 (8.5)		-1.65 (0.07)	0.19	0.03-1.11			
45-54	137 (25.5)	13 (9.5)		-1.49 (0.10)	0.27	0.04-1.40			
55-64	29 (5.4)	0 (0.0)		-20.51 (0.99)	0.00				
Education			6.7 (0.35)	0.21			0.175		
Primary	309 (57.4)	35 (11.3)			1			1	
Secondary	76 (14.1)	2 (2.6)		-1.55 (**0.04**)	0.21	0.05-0.90	-2.20 (**0.033**)	0.110	0.02-0.84
Tertiary	25 (4.6)	2 (8.0)		-0.43 (0.57)	0.65	0.15-2.90	0.45 (0.687)	1.565	0.18-13.79
never attended	128 (23.8)	13 (10.2)		-0.13 (0.71)	0.88	0.45-1.73	-0.19 (0.605)	0.824	0.40-1.71
Occupation			2.7 (0.62)	0.93			0.999		
Formal sector	35 (6.5)	3 (8.6)			1			1	
Unemployed	79 (14.7)	7 (8.9)		0.10 (0.88)	1.11	0.27-4.60	-19.32 (1.00)	-	-
Non-formal sector	424 (78.8)	42 (9.9)		0.21 (0.74)	1.22	0.36-4.18	0.06 (0.957)	1.061	0.9.32
Marital status			8.7 (0.79)	0.06			0.112		
Divorced	110 (20.4)	11 (10.0)			1			1	
Married	96 (17.8)	16 (16.7)		-0.97 (**0.01**)	0.38	0.12-0.81	-1.02 (**0.016**)	0.360	0.16-0.83
Single	206 (38.3)	15 (7.3)		-0.60 (0.15)	0.55	0.24-1.25	-0.62 (0.203)	0.539	0.21-1.40
widowed	126 (23.4)	10 (7.9)		-0.85 (0.05)	0.43	0.18-0.99	-0.66 (0.149)	0.515	0.21-1.27

Parameter	Pap smear abnormality								
	N(%)	*#n(%)*	*χ^2 (p-value)*	*β (p-value)*	*OR*	*95% CI*	*μβ (p-value)*	*μOR*	*95% CI*
Income			10.1 (0.26)	0.15			0.093		
<100	138 (25.9)	7 (5.1)			1			1	
100-249	254 (47.7)	33 (13.0)		1.07 (**0.01**)	2.87	1.24-6.69	1.16 (**0.01**)	3.20	1.33-7.71
250-500	41 (7.7)	3 (7.3)		0.42 (0.57)	1.52	0.38-6.19	0.73 (0.35)	2.07	0.45-9.46
>500	27 (5.1)	2 (7.4)		0.42 (0.61)	1.52	0.30-7.78	-0.70 (0.65)	0.50	0.03-9.96
can't tell	72 (13.5)	7 (9.7)		0.76 (0.17)	2.13	0.72-6.36	20.33 (1.00)	-	-
***Ethnicity**			15.9 (**0.04**)	0.45			0.524		
Akan	230 (43.2)	26 (11.3)			1			1	
Ewe	105 (19.7)	8 (7.6)		-0.43 (0.31)	0.65	0.23-1.45	-0.41 (0.36)	0.66	0.28-1.59
Ga	104 (19.5)	6 (5.8)		-0.76 (0.10)	0.47	0.19-1.17	-0.76 (0.13)	0.47	0.18-1.24
Mole-Dagbani	91 (17.1)	11 (12.1)		0.08 (0.84)	1.08	0.51-2.30	0.07 (0.87)	1.07	0.46-2.5
Age at maiden pregnancy			5.0 (0.54)	0.50			-0.00 (0.63)	0.99	0.99-1.00
<18	91 (16.9)	9 (9.9)			1				
18-25	333 (61.9)	36 (10.8)		0.09 (0.82)	1.09	0.50-2.37			
>25	87 (16.2)	5 (5.7)		-0.64 (0.27)	0.53	0.17-1.65			
n/a	27 (5.0)	2 (7.4)		-0.34 (0.68)	0.71	0.14-3.53			
Age at sexual debut			7.1 (0.13)	0.12			-0.09 (**0.04**)	0.97	0.84-0.99
<16	81 (15.3)	12 (14.8)			1				
16-18	247 (46.5)	23 (9.3)		-0.57 (0.14)	0.56	0.27-1.20			
>18	203 (38.2)	15 (7.4)		-0.84 (**0.04**)	0.43	0.19-0.97			

[a] Predictor variable has significant overall effect on outcome variable. * Prevalence of abnormal cytology varies significantly across categories (Pearson x^2 test).

Row percentages computed to show within-group prevalence for cervical dysplasia. Multivariate regression: Variable(s) removed in step 2: occupation. Variable(s) removed on step 3: age at first pregnancy. Variable(s) removed on step 4: ethnicity. Variable(s) removed on step 5: marital status. Variable(s) removed on step 6: age. Variables in the equation: educational status, income and age at sexual debut.

Table 4.4-2: Summary of Model Characteristics for Predicting Cytology Outcome

Model	x^2	df	P-value	Nagelkerke R^2	Variables
Step 1	35.295	19	0.013	0.141	educational status, income and age at sexual debut, age, marital status, ethnicity, age at first pregnancy, occupation
Step 2	35.000	18	0.009	0.140	educational status, income and age at sexual debut, age, marital status, ethnicity, age at first pregnancy
Step 3	34.747	16	0.004	0.139	educational status, income and age at sexual debut, age, marital status, ethnicity
Step 4	31.210	15	0.008	0.125	educational status, income and age at sexual debut, age, marital status
Step 5	25.948	11	0.007	0.104	educational status, income and age at sexual debut, age
Step 6	23.816	8	0.002	0.096	educational status, income and age at sexual debut

Table 4.4-3: Odds ratios and 95% CI for Abnormal Cytology According to Best Fit Predictive Model

Parameter	**Pap smear abnormality**			
Variable	**$^{\mu}\beta$**	**p-value**	**$^{\mu}$OR**	**95% CI**
Education		0.172		
Primary		1		
secondary	-1.671	**0.026**	0.188	0.04-0.82
Tertiary	-0.093	0.930	0.911	0.11-7.29
never attended	-0.170	0 170	0.844	0.42-1.68
Income		0.085		
<100		1		
100-249	1.249	**0.005**	3.488	1.46-8.32
250-500	0.945	0.204	2.574	0.60-11.07
>500	0.454	0.684	1.575	0.18-14.02
can't tell	1.068	0.063	2.911	0.94-8.98
Age at sexual debut	-0.117	**0.005**	0.890	0.82-0.97
Constant	0.830	0.282	0.436	

Dependent variable: Cytology outcome

Tables 4.6-4 and 4.6-5 show the crude and adjusted odds ratios (ORs) for Pap smear abnormality and corresponding 95% confidence intervals (CIs) according to behavioural and clinical characteristics of women. In univariate analysis HIV duration (months) (p=0.02), and CD4 count (p=0.04) were significantly associated with abnormal cytology outcome.

The outcome model- shown here in Tables 4.4.6-6 was based on ART use and CD4 count, significantly predicted Pap smear results (x^2 =8.46, df= 2, N=344, p=0.015) and explained up to 6% (Nagelkerke R^2=0.06) of the variability in and cytology outcome.

Table 4.4-4: Odds ratios (ORs) for cervical dysplasia and corresponding 95% confidence intervals (CIs) according to behavioural and clinical characteristics in WLHIV at the Korle Bu Teaching Hospital, Accra,

Parameter	Pap smear abnormality								
	N(%)	*#n(%)*	*χ^2 (p-value)*	*β (p-value)*	*OR*	*95% CI*	*μβ (p-value)*	*μOR*	*95% CI*
***Ever smoked**			9.9 (**0.04**)	0.58			0.15		
No	6 (1.1)	1 (16.7)			1			1	
Yes	530 (98.7)	50 (9.4)		0.61 (0.58)	1.83	0.21-16.00	-1.69 (0.15)	0.18	0.02-1.84
Current tobacco use			1.2 (0.55)	0.34					
No	534 (99.3)	51 (9.6)			1			1	
Yes	4 (0.7)	1 (25.0)		1.10 (0.34)	3.01	0.31-29.50	18.72 (0.99)	-	-
Condom use			6.7 (0.15)	0.67			0.18		
Always	145 (27.0)	12 (8.3)			1			1	
Never	256 (47.6)	25 (9.8)		0.24 (0.52)	1.27	0.62-2.61	0.43 (0.54)	1.54	0.39-6.10
Sometimes	137 (25.5)	15 (10.9)		0.36 (0.38)	1.44	0.65-3.19	1.10 (0.11)	3.00	0.78-11.51
[a] **HIV duration (months)**			12.8 (0.12)	**0.02**			-0.01 (0.48)	0.994	0.98-1.01
<48	118 (22.1)	10 (8.5)			1				
48-71	119 (22.2)	21 (17.6)		0.83 (0.04)	2.30	1.03-5.15			
72-95	104 (19.4)	7 (6.7)		-0.28 (0.59)	0.76	0.28-2.08			
96-119	106 (19.8)	6 (5.7)		-0.45 (0.40)	0.64	0.22-1.82			
>119	88 (16.4)	8 (9.1)		0.06 (0.91)	1.06	0.40-2.81			
ART use			6.2 (0.21)	0.35			0.25		
No	82 (16.0)	11 (13.4)			1			1	
Yes	429 (84.0)	41 (9.6)		-0.34 (0.35)	0.71	0.35-1.45	0.76 (0.25)	2.14	0.59-7.79

[a] Predictor variable has significant overall effect on outcome variable. * Prevalence of abnormal cytology varies significantly across categories (Pearson x^2 test).

Row percentages computed to show within-group prevalence for cervical dysplasia.

Table 4.6-4 continued

Parameter	Pap smear abnormality								
	N(%)	***#n(%)***	***χ^2 (p-value)***	***β (p-value)***	***OR***	***95% CI***	***$^{\mu}$β (p-value)***	***$^{\mu}$OR***	***95% CI***
***ART duration**			15.5 (**0.02**)	**0.01**			-0.01 (0.49)	0.99	0.97-1.02
<30	148 (34.9)	24 (16.2)			1				
30-59	95 (22.4)	4 (4.2)		-1.51 (**0.01**)	0.22	0.07-0.66			
60-89	128 (30.2)	11 (8.6)		-0.77 (**0.04**)	0.46	0.22-0.98			
>89	53 (12.5)	2 (3.8)		-1.64 (**0.03**)	0.19	0.08-0.85			
Vaginal discharge			0.8 (0.65)	0.43			0.83		
No	502 (93.5)	50 (10.0)			1			1	
Yes	35 (6.5)	2 (5.7)		-0.58 (0.43)	0.56	0.13-2.40	0.18 (0.83)	1.20	0.24-6.04
Ulcers/Sores			1.7 (0.78)	0.31			0.12		
No	527 (98.0)	50 (9.5)			1			1	
Yes	10 (1.9)	2 (20.0)		0.82 (0.31)	2.28	0.47-11.00	-1.33 (0.12)	0.27	0.05-1.44
CD4 count				-0.00 (**0.04**)	0.99	0.99-1.00	-0.00 (0.18)	0.99	0.99-1.00

[a] Predictor variable has significant overall effect on outcome variable. * Prevalence of abnormal cytology varies significantly across categories (Pearson x^2 test).
Row percentages computed to show within-group prevalence for cervical dysplasia.

Table 4.4-5: Summary of Model Characteristics for Predicting Cytology Outcome based on behavioural and clinical responses

Model	x^2	df	P-value	Nagelkerke R^2	Variables
Step 1	17.56	10	0.063	0.123	Ever smoked, current tobacco use, condom use, HIV duration, ART use, ART duration, discharge, sores, CD4 count.
Step 2	17.52	9	0.041	0.122	Ever smoked, current tobacco use, condom use, HIV duration, ART use, ART duration, sores, CD4 count.
Step 3	17.19	8	0.028	0.120	Ever smoked, condom use, HIV duration, ART use, ART duration, sores, CD4 count.
Step 4	16.81	7	0.019	0.117	Ever smoked, condom use, HIV duration, ART use, sores, CD4 count.
Step 5	15.18	6	0.019	0.106	Condom use, HIV duration, ART use, sores, CD4 count.
Step 6	13.54	5	0.019	0.095	Condom use, ART use, sores, CD4 count.
Step 7	10.14	4	0.038	0.072	ART use, sores, CD4 count.
Step 8	8.46	2	0.015	0.060	ART use, CD4 count.

Table 4.4-6: Odds ratios and 95% CI for Abnormal Cytology According to Best Fit Predictive Model based on behavioural and clinical responses

Parameter	Pap smear abnormality			
Variable	$^{\mu}\beta$	p-value	$^{\mu}$OR	95% CI
ART use	1.093	**0.025**	2.98	1.15-7.77
CD4 count	-0.002-	**0.043**	0.99	0.99-1.00
constant	-1.86	**0.000**	1.56	

Dependent variable: Cytology outcome

4.5 Correlates of high-risk HPV infection and their implications for disease prevention

In this section, the question to be addressed is which of the explanatory variables are predictive of the response, high risk HPV infection? Since the outcome was a binary categorical variable, the logistic regression model helps to determine this relationship. Therefore, investigation of the determinants of abnormal cytology proceeded in two steps. First, unadjusted relationships between high-risk HPV infection and potential predictor variables were examined singly. Table 4.7-1 shows the crude and adjusted odds ratios (ORs) for high-risk HPV infection and corresponding 95% confidence intervals (CIs) according to socio-demographic and reproductive characteristics among women. In univariate analysis, formal education and estimated income had significant main effects for high-risk HPV infection and therefore were associated with high-risk infection. This relationship did not disappear after adjusting for potential confounders such as woman's age, younger age at sexual debut and recent sexual activity. Recent sexual activity is defined for a woman who reported having a sexual partner in past six months. The regression model based on age, educational status, occupation, marital status, income, ethnicity, age at first pregnancy and age at sexual debut (Table 4.7-1), significantly predicted high risk HPV infection (x^2 =38.545, df= 15, N=427, p=0.001) and explained up to 12% (Nagelkerke R^2=0.116) of the variability in high-risk HPV infection.

Table 4.5-1: Odds ratios (ORs) for high-risk HPV infection and corresponding 95% confidence intervals (CIs) according to socio-demographic characteristics in WLHIV at the Korle Bu Teaching Hospital, Accra,

Parameter	High risk HPV infection								
	N(%)	*#n(%)*	*χ^2 (p-value)*	*β (p-value)*	*OR*	*95% CI*	*μβ (p-value)*	*μOR*	*95% CI*
Age			1.9 (0.59)	0.70			0.41		
25-34	118 (21.9)	55 (46.6)			1			1	
35-44	248 (46.1)	134 (54.0)		0.26 (0.26)	1.30	0.83-2.03	0.52 (0.12)	1.69	0.87-3.29
45-54	137 (25.5)	70 (51.1)		0.21 (0.41)	1.24	0.75-2.04	0.60 (0.12)	1.83	0.86-3.90
55-64	29 (5.4)	16 (55.2)		0.31 (0.46)	1.37	0.60-3.12	0.38 (0.57)	1.47	0.37-5.58
[a]**Formal education**			0.3 (0.56)	**0.08**			**0.03**		
Yes	410 (76.2)	214 (52.2)			1			1	
never attended	128 (23.8)	63 (49.2)		-0.37 (0.08)	0.69	0.45-1.05	-0.63 (0.03)	0.53	0.30-0.96
Marital status			7.4 (0.06)	0.09			0.89		
Divorced	96 (17.8)	47 (49.0)			1			1	
Married	206 (38.3)	95 (46.1)		-0.01 (0.97)	0.99	0.60-1.63	0.17 (0.74)	1.19	0.44-3.19
Single	110 (20.4)	58 (52.7)		0.06 (0.83)	1.06	0.61-1.86	0.22 (0.59)	1.25	0.56-2.78
Widowed	126 (23.4)	77 (61.1)		0.53 (0.05)	1.70	0.99-2.91	0.28 (0.44)	1.33	0.65-0.72

Parameter	High risk HPV infection								
	N(%)	*#n(%)*	*χ^2 (p-value)*	*β (p-value)*	*OR*	*95% CI*	*μβ (p-value)*	*μOR*	*95% CI*
[a]***Income**			21.2 (**0.00**)	**0.00**			**0.00**		
<100	138 (25.7)	93 (67.4)			1			1	
100-500	295 (54.8)	140 (47.5)		-0.96 (**0.00**)	0.38	0.25-0.58	-0.76 (**0.00**)	0.47	0.29-0.76
>500	27 (5.0)	14 (51.9)		-1.46 (0.02)	0.23	0.09-0.56	-1.62 (0.02)	0.20	0.05-0.79
Ethnicity			2.8 (0.58)	0.99			0.57		
Akan	230 (42.8)	108 (47.0)			1			1	
Ewe	105 (19.5)	55 (52.4)		0.00 (0.99)	1.00	0.63-1.61	-0.26 (0.43)	0.77	0.41-1.46
Ga	104 (19.3)	56 (53.8)		0.06 (0.81)	1.06	0.66-1.70	0.22 (0.49)	1.25	0.66-2.36
Mole-Dagbani	91 (16.9)	51 (56.0)		0.04 (0.88)	1.04	0.63-1.70	0.39 (0.28)	1.47	0.73-3.0
Age at sexual debut			0.62 (0.43)	0.73			0.75		
<18	328 (61.0)	163 (49.7)			1			1	
18 and over	203 (38.2)	108 (53.2)		0.06 (0.73)	0.73	0.75-1.52	0.07 (0.75)	1.08	0.67-1.74
Recent Sexual activity			1.9 (0.39)				0.41		
No	273 (50.7)	146 (53.3)			1			1	
Yes	264 (49.1)	130 (49.2)		-0.25(0.16)	0.77	0.55-1.10	-0.34 (0.41)	0.71	0.32-1.60

[a] Predictor variable has significant overall effect on outcome variable. * Prevalence of abnormal cytology varies significantly across categories (Pearson x^2 test).

Row percentages computed to show within-group prevalence for cervical dysplasia. Multivariate regression: Variables in the equation: age, formal education, marital status, income status, ethnicity, age at sexual debut and recent sexual activity.

Table 4.7-2 shows the crude and adjusted odds ratios (ORs) for high-risk HPV infection and corresponding 95% confidence intervals (CIs) according to behavioural and clinical characteristics of women. However, in multivariate analysis, after adjusting for age, log CD4 count and other confounders, HIV duration (p=0.04) and vaginal discharge (p=0.01) had significant main effects on cytology outcome. ART duration was not included in multivariate analysis because it was significantly correlated with HIV duration and could result in misleading variable coefficients. The final equation was based on 507 women. The resulting logistic regression equation shown here in Table 4.7-2 significantly predicts high risk HPV infection (x^2 =20.64, df= 8, N=507, p=0.008) and explained up to 5% (Nagelkerke R^2=0.05) of the variability in high-risk HPV infection.

Table 4.5-2: Odds ratios (ORs) for high-risk HPV infection and corresponding 95% confidence intervals (CIs) according to behavioural and clinical characteristics in WLHIV at the Korle-Bu Teaching Hospital, Accra,

Parameter	high risk HPV infection								
	N(%)	***#n(%)***	***χ^2 (p-value)***	***β (p-value)***	***OR***	***95% CI***	***μβ (p-value)***	***μOR***	***95% CI***
***Condom use**			5.31 (**0.02**)	**0.02**			0.07		
Always	145 (27.0)	49 (33.8)			1			1	
Never/Sometimes	393 (73.0)	176 (44.8)		-0.46 (**0.02**)	0.63	0.42-0.94	-0.40 (0.13)	0.67	0.44-1.03
[a]**HIV duration (months)**			7.92 (0.09)	0.09			**0.04**		
<48	118 (22.9)	60 (50.8)			1			1	
48-71	119 (22.1)	40 (33.6)		-0.71 (**0.00**)	0.49	0.29-0.83	-0.83 (0.00)	0.44	0.24-0.77
72-95	104 (19.3)	41 (39.4)		-0.46 (0.09)	0.63	0.37-1.07	-0.60 (0.04)	0.55	0.31-0.97
96-119	106 (19.8)	47 (44.3)		-0.26 (0.33)	0.77	0.46-1.30	-0.28 (0.34)	0.76	0.42-1.35
>119	88 (16.4)	35 (39.8)		-0.45 (0.12)	0.63	0.37-1.12	-0.58 (0.64)	0.56	0.31-1.03
ART use			0.16 (0.69)	0.69			0.60		
Yes	429 (79.7)	183 (42.7)		-0.09 (0.69)	0.91	0.56-1.46	-0.14 (0.60)	0.87	0.52-1.45
ART duration			4.33 (0.23)	0.23					
<30	148 (27.5)	66 (44.6)			1				
30-59	95 (17.7)	36 (37.9)		-0.28 (0.30)	0.76	0.45-1.28			
60-89	128 (23.8)	49 (38.3)		-0.26 (0.29)	0.77	0.48-1.25			
>89	53 (9.9)	28 (52.8)		-0.33 (0.30)	1.39	0.74-2.61			
[a]***Vaginal discharge**			5.15 (**0.02**)	**0.03**			**0.01**		
Yes	35 (6.5)	21 (60.0)		-0.79 (**0.03**)	0.45	0.23-0.91	-1.06 (**0.01**)	0.35	0.15-0.79
logCD4 count		2.64 (0.36)		-0.34 (0.24)	0.71	0.40-1.26			

[a] Predictor variable has significant overall effect on outcome variable. * Prevalence of high-risk HPV prevalence varies significantly across categories (Pearson x^2 test). # Row percentages computed to show within-group prevalence for cervical dysplasia. Categorical variables are shown as count (%).

Continuous variables are shown as mean (SD). Multivariate regression: Variables in the equation: age, condom use, HIV duration, ART use and occult vaginal discharge.

Chapter 5
DISCUSSION

Antiretroviral therapy (ART) for PLHIV provides a golden opportunity to increase cervical screening through the integration of ART services with frequent screening of women for cervical cancer (Torre *et al.*, 2016). Here, data is presented on the burden of cervical cancer and precancerous lesions among women living with HIV and attending specialized care in Accra, Ghana. HIV is known to be an important independent risk factor for development of precursor lesions and cervical cancer (Getinet *et al.*, 2015).

Although the overall prevalence of cervical epithelial abnormalities (9.3%) in this population is higher than what has previously been reported in the general population in Ghana (Odida *et al.*, 2011), no cases of squamous cell carcinoma or adenocarcinoma were detected cytologically in this population. Additionally, the prevalence rates of LSIL and HSIL are 3.9 and 2.8%, respectively. Equivocal smears (ASC-) accounted for approximately 3% of women.

These rates are higher than some estimates reported in both low-resource populations in Ghana (Handlogten *et al.*, 2014; Donkoh, 2015) and other countries with an absence of screening programs, such as Cameroun (HIV/AIDS, 2018), South Africa (Fonn *et al.*, 2015) and India (Burd, 2003b). High-resource populations with well-established screening programs and lower HIV endemicity, often have lower rates of epithelial cell abnormality (ECA). Indeed lower rates of ECA than what we found in this study have been reported among Australian (Soccio *et al.*, 2015) and Turkish women (Açikgöz and Ergör, 2010). Italian women (Meloni *et al.*, 2014) and Chinese (Tao *et al.*, 2014).

In general, studies among the general population in resource-limited countries have reported high prevalence of cervical neoplasia that coincide with estimates from HIV populations. Inadvertent inclusion of women with HIV-infection in general population studies frequently

occurs in HIV endemic populations leading to variation in estimates of squamous intraepithelial lesions (Getinet *et al.*, 2015). Clarifying the HIV status of study participants greatly helps to appreciate and compare estimates in this regard. Studies from Nigeria report ECA prevalence estimates ranging from 7.6% (Thomas *et al.*, 2012), 11.2% (Ajah *et al.*, 2015), 8.9% (De Lemos *et al.*, 2012), to 22.6% (Duru *et al.*, 2015).

Since very little is known about the prevalence and incidence of cervical abnormalities among HIV infected women and most studies in the general population reporting on cervical abnormalities do not disclose HIV status, it is plausible that high rates of ECA may reflect HIV endemicity even in general population studies. Unusually high prevalence of epithelial cell abnormalities may also be an indicator for the inclusion of HIV-infected women in the population studied (Getinet *et al.*, 2015). Previous studies with large samples of women from South African found higher rates of ECA than what is reported here: 15.2% (Allan *et al.*, 2008) and 17.3% (Richter *et al.*, 2013). Additionally, similar studies from Rwanda, 20% (Kayumba, 2014), Senegal, 21.0%, (Xi *et al.*, 2003), Guinea, 9.5% (Keita *et al.*, 2009), Gabon, 13.0%, (Assoumou *et al.*, 2016) and Ethiopia, 14.1%, (Getinet *et al.*, 2015) have all reported high prevalence of precursor cervical lesions.

Immunosuppression from HIV infection is a -known risk factor for development of precursor cervical lesions and cervical cancer and as a result, cervical lesions are expected to be more aggressively present in immunocompromised patients (Strickler *et al.*, 2005). However, the present rate of precancerous lesions falls comparably within the lower range of estimates for other high-risk (HR) populations of women. Several studies indicate that among African women, being HIV-positive was associated with a high risk of presenting squamous intraepithelial lesions of the cervix, with ORs ranging from 4.4 to 17 (Didelot-Rousseau *et al.*, 2006; Hawes *et al.*, 2006).

Precancerous cervical lesions and cervical tissue neoplasm in the cervical area were prevalent conditions in Sub-Saharan Africa. For instance, in Rwanda, a cross-sectional study of 293 HIV-infected women recruited at a military hospital found a 20.0% rate of ECA (Kayumba, 2014). Studies conducted in Zambia and Kenya reported higher diagnostic frequencies of cervical epithelial abnormalities among women infected with HIV (Lehtovirta *et al.*, 2008; Memiah *et al.*, 2012). A systematic review and meta-analysis conducted among HIV positive women in Sub-Saharan Africa showed that the prevalence of precancerous cervical lesions ranged from 2.9% to 76% (Strickler *et al.*, 2005). Furthermore, the pooled prevalence of precancerous cervical lesion among the HIV-positive women in Sub-Saharan Africa was 25.6%. In a report by Isaakidis et al., (Isaakidis *et al.*, 2013) Mumbai, cervical cytology was abnormal in 19.0% of women recruited from an antiretroviral therapy (ART) clinic. HIV positive women were four times more likely to have abnormal cytology than HIV-negative women (Blossom *et al.*, 2007). Among HIV-infected women attending Pusan National University Hospital in Korea, HIV-infected women had tenfold higher odds of abnormal cervical cytology and a prevalence of 18.3% (vs 1.8%) (Park *et al.*, 2014). In addition, studies in Cambodia (25.0%) (Marie-Eve *et al.*, 2009), the Bahamas (44.0%) (Dames *et al.*, 2009), among women attending HIV clinics also report an elevated prevalence of precancerous lesions than has been reported by this study. A comparative cross-sectional study was conducted among HIV positive women attending gynecological examination in cervical cancer screening center in Ethiopia. Prevalence of ECA (17.8 %) was observed among HIV positive women (Getinet *et al.*, 2015).

Sub-Saharan Africa showed that the prevalence of precancerous cervical lesions ranged from 2.9% to 76% (Strickler *et al.*, 2005). Among HIV-infected women attending Pusan National University Hospital in Korea, HIV positive women had tenfold higher odds of abnormal cervical

cytology and a prevalence of 18.3% (vs 1.8%) (Park *et al.*, 2014). In addition, studies in Cambodia (25.0%) (Marie-Eve *et al.*, 2009), the Bahamas (44.0%) (Dames *et al.*, 2009), among women attending HIV clinics also report an elevated prevalence of precancerous lesions than has been reported by this study. A comparative cross-sectional study was conducted among HIV positive women attending gynecological examination in cervical cancer screening center in Ethiopia. Prevalence of ECA (17.8 %) was observed among HIV positive women (Getinet *et al.*, 2015).

HIV is known to be an important independent risk factor for development of precursor lesions and cervical cancer (Getinet *et al.*, 2015). Antiretroviral therapy (ART) for PLHIV provides a golden opportunity to increase cervical screening through the integration of ART services with frequent screening of women for cervical cancer (Torre *et al.*, 2016). Early initiation of HAART and sustained adherence for viral control is likely to reduce incidence and progression of SIL and ultimately incidence of invasive cervical cancer (Memiah *et al.*, 2012; Akarolo-Anthony *et al.*, 2013). The lower threshold of cervical pre-cancer/SIL in the presence of HPV infections among women living with HIV may reflect the high coverage of HAART in this population (80%) even though data on the length of ART was not available, it is expected to be high among women recruited from HIV clinics such as this (Minkoff *et al.*, 2010; Massad *et al.*, 2016). Several African studies (Clifford *et al.*, 2006d; Chaturvedi, 2010; Doorbar *et al.*, 2012; Zeier *et al.*, 2012) provide indication that earlier initiation and effective HAART over a prolonged duration can prevent cervical lesion incidence and progression and promote regression. Recent evidence from two systematic reviews and meta-analyses of studies in African populations and the world over have concluded that early initiation of HAART coupled with a high HAART compliance may be the defining reason for lower-than-expected prevalence of precancerous and cancerous lesions in hospital-based studies among women with HIV (Harms *et al.*, 2006; Akarolo-Anthony *et al.*, 2013).

Although the Pap Smear test has a very good specificity for malignancy and precursor lesions, it suffers a corresponding low sensitivity at the same time (Saslow *et al.*, 2012). The implication of this may be that a high proportion of genuine cases may be missed, usually showing up in cytology reports in the ambiguous atypical squamous cells (ASC) category, with adverse clinical implications. The results of this study confirm that ASC were a common finding (Davey, 2005). The most effective way to manage this outcome is a matter of concern for healthcare services. Recent guidelines recommend that, following two initial normal Pap-smears at a 6-month interval, all HIV-positive women should undergo annual cervical cytologic examination. In addition, it is recommended that all immunosuppressed women with atypical squamous cells undergo colposcopy (Yar *et al.*, 2016). In well-endowed settings, cervical cancer screening strategies integrating HPV testing have been proven to improve disease detection and afford longer screening cycles (Saslow *et al.*, 2012) and could be explored in high-stakes patient populations such as this. This approach benefits from the HPV test's greater sensitivity in diagnosing women with CIN3+ (Arbyn *et al.*, 2006b). In more advanced cytology screening settings, the practice has been to perform reflex HPV testing for all ambiguous cytology reports (ASCUS) and onward colposcopy evaluation in the event of a positive HPV screen. Another way is to perform cytology and HPV testing simultaneously (Wright *et al.*, 2014). Both approaches have been shown to improve the clinical performance of cytology in other settings. Though several studies have consistently shown a high burden of HPV-associated diseases in HIV positive women even in the era of HAART (Sinayobye *et al.*, 2014), presently, there is limited or non-existent routine screening services for many HIV positive women. There are advantages to providing routine screening to HIV positive women. In Zambia, for example, routine screening prevented one death from ICC for every 46 HIV positive women screened (Yar *et al.*, 2016).

Cervical cancer caused by persistent infection with high-risk human papillomaviruses is a defining condition of HIV/AIDS.(Denny *et al.*, 2012) In the present era of type-specific vaccines and HAART, there is a lot of interest regarding the prevalence and distribution of HPV among women living with HIV. This study presents an estimate on the fraction of individuals carrying a new, persistent, or recurring HPV infection in the population of women presenting to HIV clinic during the study period. Among women enrolled in this study, the overall prevalence of HPV infection was 52.0% and the prevalence of high and low risk HPV infection was 29.7% and 10.2% respectively. Among women on ART the overall prevalence of high-risk HPV infection was 80.3% against 91.7% in those not enrolled on ART.

Concordant estimates have been reported in several studies from sub-Saharan Africa. A cross-sectional hospital-based study involving 255 HIV infected women was conducted at an HIV care and treatment clinic in Mwanza, Tanzania. The prevalence of HPV among HIV positive women was 51.4% while the prevalence of high risk types was 48.6% (Mbulaiteye *et al.*, 2006). Among 220 HIV positive women recruited during community cervical cancer screening programme in states of Ogun and Lagos, the prevalence of only high risk HPV infection was 24.5% (Gelband and Sloan, 2007) similar to what was found in the present study. In a cross-sectional observational study among HIV positive women recruited at the University Teaching Hospital in Lagos, Nigeria, the prevalence of HPV among HIV positive women was 44.9% while the prevalence of high risk types was 37.5% (Franceschi and Jaffe, 2007).

The present findings (prevalence of 52.0%) are in line with the results from other studies in other countries that reported high HPV prevalence rates in HIV-infected women (Chuang, 1987; De Sanjosé *et al.*, 2007; Vera-Iglesias *et al.*, 2007; Bedoui *et al.*, 2009). Park et al., (2007) retrospectively reviewed women living with HIV attending Pusan National University Hospital

(Korea) for the prevalence and genotype distribution of HPV infection and reported an overall HPV infection rate of 46.7%.

Highly elevated rates of HPV infection have been reported in the Ashanti Region of Ghana and in other major cities in African (De Vuyst *et al.*, 2009; Gasparini and Panatto, 2009). Yar *et al.*, (2015) conducted a study on 107 women infected with HIV. Overall HPV positivity was 86.9%. The excess HPV positivity was probably an effect of the expanded number of 28 high- and low-risk HPV genotypes that were tested for compared to 18 HPV genotypes in the present study. High prevalence rates of HR-HPV have been reported among two separate studies in HIV-positive Rwandan women in Kigali, 46% (Trottier *et al.*, 2006a) and 50.8% (De Sanjosé *et al.*, 2007) as well as studies in other double risk populations such as commercial sex workers infected with HIV with reported HR-HPV prevalence rates ranging from 44-73% (Trottier and Franco, 2006; Trottier *et al.*, 2006a; Luchters *et al.*, 2010), In all of these studies, the ART status of participating women was undisclosed and could point to the higher than expected rates. In a cross-sectional study of 498 HIV-positive women in Kenya, 68.7% of women were HPV-positive: and 52.6% had HR HPV. Elevated CD4 count and prolonged HAART use were significantly associated with lower prevalence of high-risk HPV infection in that study (Gasparini and Panatto, 2009).

This prevalence of 68.7% is considerably higher than the prevalence rates of 37.2% that was found in women from the general population in Kumasi, Ghana (Donkoh, 2015). It is well established that there is a significantly higher HPV prevalence in HIV positive women compared to HIV negative women. There are several comparative studies to support this claim. A high magnitude of association of HIV infection with high-risk HPV was previously found in a Uganda (Bosch *et al.*, 1992) and Rwanda (Franceschi *et al.*, 2006). Park *et al.*, (2014) retrospectively reviewed HIV-infected women attending a University Hospital for the prevalence and genotype distribution of HPV infection among HIV-infected women in Korea.

The authors of the study reported an overall, HPV infection rate significantly higher and associated with five-fold odds in HIV-positive women than HIV-negative women (46.7% against 14.1%). A prevalence of (77.4% against 41.6%) Yar *et al.*, 2016 and (65.6% against 30.2%) Obiri-Yeboah *et al.*, 2017 in HIV-positive and HIV-negative women respectively were observed in studies in Ghana (Yar *et al.*, 2016; Obiri-Yeboah *et al.*, 2017). Other studies previously showed that HIV-infected women are more likely than those without HIV infection to have persistent HPV (Adler *et al.*, 2015)

In general, the significantly higher HPV prevalence among HIV-positive women can be attributed to increased susceptibility, decreased ability to clear infection which may be due to factors directly related to immunosuppression and frequent reactivation of latent infection associated with immunosuppression (Khan *et al.*, 2005). The destruction of CD4 cells by HIV may increase the likelihood of HPV establishing infection (Mbulaiteye *et al.*, 2011). The evidence also suggests that immunosuppression leads to higher probability of HPV reactivation, potentially due to incomplete clearance of HPV DNA (Humans *et al.*, 2007). In one African study HPV was inversely associated with age in HIV negative women as the prevalence of HPV among HIV positive women remained high in all age-groups (Bosch *et al.*, 1992).

Owing to advances made in ART and management, women infected with HIV now live longer, fulfilling lives. Cervical cancer caused by HPV is considered to be a needless and preventable cause of death. Knowledge of the distribution of HPV genotypes prevalent among high-risk populations can guide the implementation of successful primary prevention of these cancers in women who eventually develop HIV infection. The commonest HPV types detected here were HPV-35 (14.1%), HPV-18 (13.0%), HPV-58 (7.4%), HPV-52 (7.2%), HPV-45 (4.8%) HPV-44 (3.7%), HPV-39 (3.3%) and HPV-68 (3.2%). The frequency of HPV 16 was 2.4%. HPV 42, a low-risk type, was also common (7.8%).

Although the mechanism by which HIV increases risk of cervical cancer is not completely understood, studies suggest that HIV-induced immunosuppression leads to an inability to control the expression of HPV and the production of HPV oncoproteins E6 and E7 (McKee *et al.*, 1981) and the risk appears to be associated with increased HPV persistence that may result from immunosuppression related to HIV. Furthermore, the risk is greater in women with CD4 counts less than 200 cells per microliter and in those with high plasma HIV RNA levels (Hawes *et al.*, 2006). There is evidence that HIV-1 infection is associated with an increased rate of HPV infection, mainly restricted to HR-HPV types which are the cause of invasive cancer of the cervix (Didelot-Rousseau *et al.*, 2006; Petry *et al.*, 2012).

There are not many reports on the molecular epidemiology of HPVs in Ghanaian women. Furthermore, differences in analytical methodology and sensitivity complicates any potential comparisons. A study involving HIV women in Kumasi showed that infections of HPV 58, 35, 31, 68, 53, 52, 18, 16 were most common (Yar *et al.*, 2015). Apart from variability in HPV 31 and 53, the rest of the HPVs detected were confirmed in this study as well to be prevalent among Ghanaian women with HIV infection. The common genotypes observed in this study was also similar to a study conducted in Central Region of Ghana (Obiri-Yeboah *et al.*, 2017) where the authors found (HPV 35, 52, 58, 16 and 18)).

HIV-infected women are reported to acquire a broader spectrum of HPV genotypes compared to HIV-naïve women (Clifford *et al.*, 2006c; Didelot-Rousseau *et al.*, 2006; Sahasrabuddhe *et al.*, 2007). Among populations of HIV positive women in the same country, diverse HPV genotypes may be prevalent depending on several potential confounders such as ART regimes and sexual activity of women. In a previous studies evaluating the concordance of HPV infection among heterosexually active couples and the impact of HIV coinfection on the prevalence of HPV, HIV coinfection in one partner had a significant impact on the prevalence of HPV infection in the other partner (SCHEURER *et al.*, 2005). Concordance of the same HPV genotypes was more

commonly found among couples where one or both partners were HIV-infected, compared with HIV-uninfected couples (SCHEURER *et al.*, 2005). The majority of WLHIV got infected by their male sexual partners in Korea (SCHEURER *et al.*, 2005). This can be very significant for cervical cancer prevention in this population.

In a comparative cross-sectional analytic observational study among HIV positive women at the Lagos University Teaching Hospital (LUTH) Lagos, Nigeria, the commonest high risk types seen were types 31, 52, 53 and 35 (Franceschi and Jaffe, 2007). In a cross-sectional study involving 106 women at an STI clinic in Kampala the commonest types were HPV 52, HPV 16, and HPV 58; HPV 16/18 combined was 18.4% (Blossom *et al.*, 2007). In a cross-sectional study of 498 HIV-positive women in Kenya, common genotypes found included HPV-35 (12.3%), HPV-16 (10.8%), HPV-52 (7.2 %), HPV-56 (6.4 %), HPV-18, 58 (5.4% each), and HPV-31 (5.2%) (Gasparini and Panatto, 2009).

Park et al. (2014) retrospectively reviewed HIV-infected women attending Pusan National University Hospital in Korea and observed that, the most common HR-HPV genotype detected in these women was HPV 16 (10%), followed by 18 (6.7%) and 52 (5%). However, this was a limited sample size. Prevalence of quadrivalent vaccine-preventable types (HPV 6, 11, 16, and 18) was 21.7% and 2.3% in HIV-positive women and HIV-negative women, respectively. The results of the present study suggests that there may be some variability in the distribution of high-risk genotypes in HIV positive women compared to the general population as noted previously (Yar *et al.*, 2015). The detection of all HR types, even HPV16, is higher in HIV positive women in comparison with HIV-negative women. However, the prevalence of HPV16 in HIV positive women is known to be less affected by decreases in CD4+ cell counts than other high-risk HPV types (Wanichwatanadecha *et al.*, 2012). This may occur because clearance of oncogenic HPV infection is less successful in HIV positive women due to a lack of an appropriate immune response (Frisch *et al.*, 2000) which may be especially obvious for high

risk genotypes other than 16 and 18 (Bernard *et al.*, 2010). These two are thought to be sufficiently oncogenic that their persistence in the cervical mucosa is less dependent on the immune system of the host woman. Once the impact of ART in HPV infection and cervical cancer is defined, the dynamics of this variability would be easier to appreciate.

The combined prevalence of 15.4% of the highly oncogenic HPV 16 and 18 in this study is similar to estimates in other studies among HIV positive women and in the general population (Münger *et al.*, 1992; Clifford *et al.*, 2006a). The reason may be that HPV 16 and 18 are better at evading the host immune system and are less impacted by HIV-associated immunosuppression (Boshart *et al.*, 1984; Cogliano *et al.*, 2005). In one case, the relative risk of acquiring HPV 16/18 was higher among HIV-positive women, nevertheless HPV 16/18 clearance was not found to differ by HIV status (Bernard *et al.*, 2010; Wanichwatanadecha *et al.*, 2012). Studies have found that HPV 16 in particular is more weakly associated with the immune system compared to other types (Münger *et al.*, 1992; Wanichwatanadecha *et al.*, 2012). This is consistent with studies that find HIV-positive women have a greater diversity of HPV types in normal cytology and low-grade lesions, but the relative prevalence of HPV 16 and 18 increases with severity of lesions and CC and the proportion of cervical cancer attributable to HPV 16/18 is similar between HIV positive and negative women (Jeon *et al.*, 1995; Forslund *et al.*, 2004).

Quadrivalent HPV vaccine was immunogenic and well tolerated in HIV-infected young women in a study (Palefsky *et al.*, 1999). HPV vaccine is recommended by the Advisory Committee on Immunization Practices (ACIP) for HIV-infected individuals through age 26 years for those who did not get any or all doses when they were younger (Bernard *et al.*, 2010). Prevalence of vaccine-preventable types (HPV 6, 11, 16, and 18) or types (HPV 6, 11, 16, and 18, 31, 33, 45, 52, 58) in our HIV-infected women was 21.3% and 43.3%% respectively, suggesting that innovative strategies would be needed to effectively prevent these infections. HPV DNA or

serologic screening may be needed before immunization in HIV-infected women in Ghana, although the ACIP does not recommend HPV DNA or serologic screening before immunization (Bernard *et al.*, 2010).

It is well established that the contribution of different HPV genotypes to cervical cancer and intraepithelial lesions vary greatly owing to differences in carcinogenic potential (Muñoz *et al.*, 2004). However, HPV infection in general progresses to squamous intraepithelial neoplasia more frequently and rapidly in HIV-infected women than in the general population (Boshart *et al.*, 1984; Münger *et al.*, 1992; Organization and Cancer, 2007). This work presents the relationship between detection of HPV genotypes and the presence of cytological lesions in women living with HIV (Table 4.5-1) to give insight on the impact of HIV-related immunodeficiency on the natural history of high risk HPV in incidental cervical precancerous lesions. Prevalence of HPV infection ranged from 25.9% among women with normal Pap findings to 53.3% among women with ASCUS. No significant differences were noted for the nature of the infection: any type HPV infection, high-risk HPV, or low-risk HPV and Pap smear outcome. The commonest HPV genotypes detected among women with ASCUS were HPV-18, 35 and 31, 39, 45, 51, 52, 58, 68. Among women with LSIL most prevalent were HPV-52, HPV-35, HPV 16 and HPV 18, 58, 39. HPV-52, 35 and 58, 45 were top three in that order for high grade intraepithelial (HSIL)/severe dysplasia lesions. The genotype-specific distribution detected in women without any precancerous lesions was as follows: HPV-35, HPV-18, HPV-52, HPV-58 and HPV-68.

The result of the present study confirms that there is an increased attributable fraction for non-HPV16 types in precancerous lesions. This relative shift from HPV16 to other HR types has been observed previously for HIV-infected women with normal cytology and cervical intraepithelial lesions (Clifford *et al.*, 2006a), and has significant implications for using vaccine-

based prevention strategies as a sole measure among women with HIV. A meta-analysis confirmed that HPV16 was underrepresented relative to other high-risk HPV types among HIV-positive women, in all grades of cervical diagnoses up to high-grade precancerous lesions (Clifford *et al.*, 2006a). Variation in HPV genotype distribution in ICC is not new as it has been observed in other regions of the world. It is hypothesized that host immunogenetic factors and biologic interplay between different HPV genotypes or variants are most likely responsible (Bousarghin *et al.*, 2003).

Studies from sub-Saharan Africa have shown under-representation of HPV-16 in women with abnormal cytology that have been attributed to HIV/AIDS endemicity and the propensity for women with HIV to harbor a greater range of HPVs including multiple infections (Boshart *et al.*, 1984). HPV 35, for instance, was slightly more common than HPV 16 in Mozambique both in women with normal cytology and in those with HSIL or worse (Castellsague *et al.*, 2001). HPV 52 was found slightly more frequently than HPV 16 or HPV 35 in Kenya (De Vuyst *et al.*, 2003) and in women with normal colposcopy in Zimbabwe (Gravitt *et al.*, 2002). In Senegal, HPV 16 and 58 were the most common types overall and in women with cervical lesions (Xi *et al.*, 2003). Indeed, the importance of HPV16 increases with severity of lesion (Muñoz *et al.*, 2004), so that HPV16 remains by far the most common high-risk HPV type, in both HIV-positive and HIV-negative ICC (Boshart *et al.*, 1984). However, the proportion of ICC attributable to HPV16 is marginally lower in HIV-positive than HIV-negative women. This shows that it will be useful to focus preventive effort on other HR types for women with HIV in order to prevent the occurrence of cervical intraepithelial lesions. This effort may not be as beneficial in ICC since the difference in HPV16 proportion in ICC is less dependent on HIV status than that seen among normal cytology and cervical intraepithelial lesions (Clifford *et al.*, 2006a).

Nevertheless, the present data confirms reports that the attributable fraction for other vaccine types such as HPV18, 52 and 45 in cervical pre-cancer is concomitantly higher, so that current expanded prophylactic vaccines against these genotypes may be of greater utility in HIV positive women than earlier HPV16 and 18 based vaccines, which have shown some promise anyway in HIV-infected populations (Frellick, 2017).

In general, the high prevalence of high-risk HPV in ASCUS/LSIL and HSIL among HIV positive women in the present study is similar to several reports on HIV-positive women with precancerous lesions. Concomitant reports of a high prevalence of high risk HPV genotypes have been documented in sub-Saharan Africa in cervical/vaginal specimens and HSIL lesions (Blossom *et al.*, 2007; Donkoh, 2017; Donkoh *et al.*, 2019a). In these reports, HIV positive women had more high risk HPV genotypes detected compared to HIV negative women. In a particular report, mean number of HPV genotypes detected was higher among HIV positive compared to HIV negative women (Donkoh *et al.*, 2019a).

The high prevalence of non-vaccine preventable genotypes such as HPV-35 in HSIL in this study is an important finding because of implications for disease prevention. Many studies report a high prevalence of HPV 35 in Africa. For instance, it was slightly more common than HPV 16 in Mozambique both in women with normal cytology and in those with HSIL or worse (Castellsague *et al.*, 2001). Data from meta-analysis of 21 African studies reveal that a large part of the non-vaccine-type related ICC remaining fraction in African women is attributable to HPV35, regardless of HIV status (Boshart *et al.*, 1984).

Three prophylactic vaccines containing virus-like particles that offer protection against cervical pre-cancers and cancers are available. Studies have indicated that these vaccines are safe, well tolerated and efficacious against the vaccine-related HPV types that cause persistent infection

and cervical disease in young ladies (Harper *et al.*, 2004; Lu *et al.*, 2011; Petrosky *et al.*, 2015). Additionally, the quadrivalent and nonavalent vaccines targeted against HPV 6, 11, 31, 33, 45, 52 and 58 and can prevent 90% of all cases of genital warts (Villa *et al.*, 2005; Petrosky *et al.*, 2015). Mathematical models predict that widespread use of preventive HPV vaccines containing genotypes 16/18 have the potential to reduce deaths from ICC by 50% over several decades (Koutsky and Harper, 2006; Nobre *et al.*, 2009).

In sum, the major study finding is that high-risk HPV infection was frequent in women living with HIV and vaccine preventable HPVs are significantly associated with squamous intraepithelial lesions, suggesting that this population follows an epidemiological pattern in common with other HPV-infected women, who remain at high risk for cervical neoplasia from high risk, vaccine preventable genotypes. Additionally, there was a high prevalence of non-vaccine HPV types such as HPV-35, 39 and 68 among women with ambiguous and high grade squamous intraepithelial lesions. As such, early vaccination may protect women who eventually develop vaccine-preventable HPV types but for best results, regular screening of women and colposcopic management of HIV-positive women will continue to be justified for preventing the excess morbidity from non-vaccine types found here.

HIV is known to be an important independent risk factor for development of precursor lesions and cervical cancer (Getinet *et al.*, 2015). Several studies conducted in sub-Saharan Africa indicate that among African women, being HIV infected was associated with a high risk of presenting squamous intraepithelial lesions of the cervix, with ORs ranging from 4.4 to 17 depending on the grade of the lesion and other factors (DiMaio and Petti, 2013; Doorbar, 2013). Low- and middle-resource countries, where women have been hit hardest by the AIDS epidemic, have historically also had very high prevalence of human papillomavirus (HPV) infection (Clifford *et al.*, 2006b) and a high incidence of cervical cancer and precancerous

lesions (Parkin *et al.*, 2003). With the adoption of HAART, women living with HIV and persistent HR-HPV are expected to live longer than before and may develop precancerous lesions which, if unchecked, may retrogress to end-stage cancer (Moodley and Mould, 2005). This study investigated the determinants of abnormal cytology among women living with HIV and attending specialized care in Accra, Ghana. After adjusting for potential confounders such as woman's age and age at first pregnancy, age at sexual debut (mean 18.3 ± 3.0 years) had significant main effect for abnormal Pap smear result later in the life of women. This finding is supported by the report from Ethiopia (Getinet *et al.*, 2015) and Tanzania (Gondos *et al.*, 2005). The final predictive model was based on educational status, income, and age at sexual debut, significantly predicted Pap smear results explained up to 10% of the variability in cytology outcome (Table 4.6-3). Educational status, income, and age at sexual debut (Gondos *et al.*, 2005; Getinet *et al.*, 2015) have been previously reported to be important predictors of precancerous lesions in similar studies. Other important risk factors that have been reported such as marital status (Getinet *et al.*, 2015), polygamy, history of STI (Gondos *et al.*, 2005; Getinet *et al.*, 2015; Schulze *et al.*, 2016), oral contraception and high parity (Getinet *et al.*, 2015) were not significant risk factors in the present study. The reason for this might be that the effect of some variables may be modified by other variables and possibly confound their effect in different populations (Gondos *et al.*, 2005; Getinet *et al.*, 2015).

Among women with normal cytology and throughout the range of cytological abnormalities detected in African women HPV genotype distribution often differs from other world regions (WHO/ICO, 2010b). Variation in HPV genotype distribution in cervical lesions results from host immunogenetic factors and biologic interplay between different HPV genotypes (Bousarghin *et al.*, 2003). Since this variation has implications for the efficacy of multivalent preventive HPV vaccines in high-risk populations, there is much interest to decipher the putative

factors predicting high-risk HPV infection in these populations such as HIV positive women in particular. Regarding socio-demographic characteristics, after adjusting for potential confounders such as woman's age, younger age at sexual debut and recent sexual activity, formal education and income status were associated with high-risk infection (Table 4.7-1). Regarding women's sexual behavioral characteristics, after adjusting for potential confounders such as age and log CD4 count, duration of HIV infection (p=0.04) and vaginal discharge (p=0.01) were significant predictors of cytology outcome (Table 4.7-2).

In a similar study, 498 HIV-positive women were assessed for the cross-sectional association of HPV and CIN with various characteristics, CD4 count and use of combination antiretroviral therapy (cART) among HIV-positive women. High-risk human papillomavirus-positivity did not vary significantly by age but it was negatively associated with education level (Gasparini and Panatto, 2009).

The finding that duration of HIV infection is a predictor of HR-HPV infection confirms what has been reported previously and may explain how the young age and HIV positivity may interact in the aetiology of cervical lesions (Palefsky *et al.*, 1999). In a recent systematic review of twenty studies in African women, it was observed that "number of lifetime sexual partners", "HIV infection" and "age" were the risk factors for high occurrence of HPV infection which is consistent with other studies (Palefsky *et al.*, 1999)The number of sexual partners which has often been cited as a risk factor for high-risk HPV infections (Franceschi *et al.*, 2006) was not associated with high-risk HPV here. Hormonal contraceptive use was also not found to be associated with high-risk HPV infection among women in conformity with other studies in African women (Lazcano-Ponce *et al.*, 2001; Peyton *et al.*, 2001; Wandeler *et al.*, 2016). Rather, a weak association between barrier method of contraception and high-risk HPV became insignificant after adjusting for HIV duration in this study.

ART duration was not an independent predictor of high-risk HPV prevalence but its effects were likely mediated through HIV duration. This was confirmed by (Franceschi *et al.*, 2006) in similar circumstances in Rwanda. Although prophylactic HPV vaccines may prove to be the ultimate cervical cancer prevention strategy, there are already 2-3 generations of at-risk, high-risk HPV-positive women who will not benefit from HPV vaccination, and universal HPV vaccination is decades away. Moreover, in the ART era, HIV positive women will live longer, have a greater high-risk HPV burden, and in the absence of comprehensive screening and treatment, will likely remain at an elevated risk of invasive cervical cancer. Thus, there is still a need to better understand the hallmarks of natural immunity against HPV infection. This may provide therapeutic avenues against HPV and HPV related disease that might be used to prevent HPV-related cancers in those already infected with high-risk HPV

Chapter 6
CONCLUSIONS

The prevalence of precancerous cervical lesions was lower than what has been reported among other cohorts of HIV-infected women in Africa and similar settings. Although the prevalence of precancerous cervical lesion and tissue change reported among HIV-positive women was below regional estimates in this population, it was still very high and represents a significant proportion of the clinic population that would benefit from screening and treatment to prevent invasive cancer.

Hence, this finding strongly confirms that HIV-infection is highly convergent with development of a cervical precancerous lesions and has implication for the management of HIV women attending specialized healthcare in the country especially in the era of sustainable ART. It is recommended that screening of HIV-infected women for cervical cancer should be a matter of routine practice. Availability and accessibility of these services can be done through their integration into HIV clinic manuals. Prompt initiation of HAART through an early enrolment into care may reduce the prevalence and progression of cervical precancerous lesions. Furthermore, this finding has implications for future interventions in the implementation of prophylactic HPV vaccines to be the part of care and support programme for HIV-infected women according to recommended guidelines for extended vaccination in sexually active HPV negative women.

In conclusion, HIV positive women had a high burden of HPV infection at the time when their life-spans are being prolonged by expanded access to highly active anti-retroviral therapy (HAART) and medical care. Though several studies have consistently shown a high burden of HPV-associated diseases in HIV positive women even in the era of HAART, presently, there is limited or non-existent routine screening services for many HIV positive women. Evidence elsewhere demonstrates that there are advantages to providing routine screening to HIV positive

women. In Zambia, for example, routine screening prevented one death from ICC for every 46 HIV positive women screened In the United States, CC screening programs have been highly effective in reducing CC incidence and HPV vaccines have been shown to reduce the prevalence of HPV 16 and 18-attributable precancerous lesions.

The high prevalence of HPV infection, STIs, and cervical lesions detected among women attending ART clinic in this study demonstrates a need for routine cervical screening of all HIV-infected women in this setting. These patients need to be properly triaged in order to ensure effective use of scarce resources. In the meantime, a simple, preferably one-stop, cervical cancer screening strategy may be a sustainable model for HIV positive women.

The results of this study confirms findings that there may be some variability in the distribution of HPV genotypes in HIV positive women compared to the general population. The potential spill-over effects of genotypes from this high-risk population into the general population may be significant for cancer prevention efforts in the Ghanaian population. In this study, HIV positive women had multiple HR-HPV genotypes in excesses of HPV 16/18 consistent with previous studies. This finding is in line with reports that the reason HPV 16 and 18 are underrepresented in precancerous lesions compared to other genotypes may be that, they are better at evading the host immune system and are less impacted by HIV-associated immunosuppression. Consequently, it is clear that the vaccines with expanded genotype coverage may be more beneficial than those containing only HPV 16 and 18 genotypes in preventing precancerous lesions in this population. It is also clear that cervical screening will continue to play a role in cancer prevention in this population.

In sum, the major study finding is that high-risk HPV infections were frequently detected in women living with HIV infection. Given the high prevalence of HIV-HPV coinfection, associated cervical lesions represent a major public health burden in the country. Vaccine preventable HPVs are significantly associated with squamous intraepithelial lesions, suggesting

that this population follows an epidemiological pattern in common with other HPV-infected women, who are at high risk for cervical neoplasia from high risk, vaccine preventable genotypes.

HR-HPV 16, 18, 31, 33, 45, 52 and 58 targeted by two licensed preventive HPV vaccines are the most common genotypes found in invasive cervical cancer even among HIV positive women. However, the present data reveals that a large number of women may harbour other HR-HPV genotypes not targeted by the currently available HPV vaccines: there was a high prevalence of non-vaccine HPV types such as HPV-35, 39 and 68 among women with ambiguous and high grade squamous intraepithelial lesions. The indication is that these vaccines could be less effective and leave women open to risk for invasive cancer if used in isolation.

Early vaccination may protect women who eventually develop vaccine-preventable HPV typeks but for best results, regular screening of women and colposcopic management of HIV-positive women will continue to be justified for preventing the excess morbibity from non-vaccine types found here. Moreover, in the future, any preventive HPV vaccine(s) should contain more HR-HPV genotypes of significance confirmed by more evidence.

In conclusion, it was established that early age at sexual debut was a significant risk factor for precancerous lesions among HIV positive women. Therefore, awareness creation on these risk factors such as early age of sexual debut and those reported in other studies should be intensified as part of preventive effort. The general awareness of risk factors of cervical dysplasia is very low even among well-educated women. Furthermore this study proves that such awareness should be provided to girls at the level of primary education in order to be most effective. The predictive model shown here (Table 4.6-3) has important implications for the management of HIV-positive women and other high-risk populations in the era of HAART.

In conclusion, we found that HIV positive women presenting for routine clinical care had a high high-risk HPV prevalence. The present data have important implications for the management of

this common occurrence of high-risk HPV in HIV-positive women and other high-risk populations in the era of HAART. In the general population, high-risk HPV-based screen and treat, in which all high-risk HPV-positive women are treated immediately, may be a reasonable strategy, despite the lack of adequate colposcopy facilities and pathologists to provide tissue-based diagnosis to guide treatment. However, in the HIV positive population, especially those who are highly immune suppressed and are likely to be at the greatest risk of cervical cancer, the data available here shows that high-risk HPV-based screening may not have the desired specificity to be used alone, especially in a screen-and treat strategy. In the current context, approximately a third of the HIV positive women would test high-risk HPV positive and be treated. In other populations, even greater proportions would be treated Thus, it may be desirable to use a secondary, triaging formula to determine which high-risk HPV-positive women need immediate treatment (triage positive) and which might have treatment deferred (triage negative) until there is evidence of high-risk HPV persistence.

Study Limitations

This study had limitations mainly due to the cross-sectional design with potential problems with direction of causality. Eighteen HPV genotypes were tested for in this study, we have approximately 40 alpha-papillomaviruses capable of infecting the ano-genital tract of human. We did not sequence the genotypes to check on gene variability.

6.1 RECOMMENDATIONS

Epithelial cell abnormalities usually develop without recognizable symptoms. Therefore the rationale for screening for cervical cancer has always been to detect abnormal cervical cells: including precancerous cervical lesions, as well as early cervical cancers at an early stage where

remedial measures may be taken at reasonable cost. Most women in countries with poorly-resourced healthcare systems do not have access to reliable screening programs and consequently report to hospital only when lesions are at an advanced invasive stage. Women living with HIV are not excluded from this accessibility challenge. Overall screening and diagnosis is a major challenge in developing countries, Ghana inclusive. In the light of the findings of this work, this situation must begin to change for the better. **Access to reliable cervical cancer screening must be considered to be a priority for high-risk populations.**

This work has confirmed previous reports in overseas settings that women living with HIV have a greater prevalence of precancerous lesions by demonstrating a similar trend in a population of women from the largest Ghanaian HIV clinic. **It is recommended that focus must be given to treating women with precancerous lesions to prevent their progressing to cervical cancer and death.** Further investigations are need to determine the optimal cervical cancer screening strategies, those that balance the benefits and harms of screening, in known HIV-positive women living in Africa.

REFERENCES

Açikgöz A. and Ergör G. (2010) Cervical cancer risk levels in Turkey and compliance to the national cervical cancer screening standard. *Asian Pacific journal of cancer prevention: APJCP* 12, 923-927.

Adanu R.M. (2002) Cervical cancer knowledge and screening in Accra, Ghana. *Journal of women's health & gender-based medicine* 11, 487-488.

Adebamowo S.N., Olawande O., Famooto A., Dareng E.O., Offiong R., Adebamowo C.A. and Group H.A.A.R. (2017) Persistent low-risk and high-risk human papillomavirus infections of the uterine cervix in HIV-negative and HIV-positive women. *Frontiers in public health* 5, 178.

Adebamowo S.N., Olawande T., Famooto A., Dareng E. and Adebamowo C. (2016) Incidence, clearance and predictors of type-specific HPV infection among Nigerian women: American Society of Clinical Oncology.

Adler D., Wallace M., Bennie T., Abar B., Sadeghi R., Meiring T., Williamson A.-L. and Bekker L.-G. (2015) High risk human papillomavirus persistence among HIV-infected young women in South Africa. *International Journal of Infectious Diseases* 33, 219-221.

Agorastos T., Dinas K., Lloveras B., de Sanjose S., Kornegay J.R., Bonti H., Bosch F.X., Constantinidis T. and Bontis J. (2005) Human papillomavirus testing for primary screening in women at low risk of developing cervical cancer. The Greek experience. *Gynecologic Oncology* 96, 714-720.

Agyei-Mensah S. and Aikins A.d.-G. (2010) Epidemiological transition and the double burden of disease in Accra, Ghana. *Journal of urban health* 87, 879-897.

Ahdieh L., Klein R.S., Burk R., Cu-Uvin S., Schuman P., Duerr A., Safaeian M., Astemborski J., Daniel R. and Shah K. (2001) Prevalence, incidence, and type-specific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. *Journal of Infectious Diseases* 184, 682-690.

Ajah L.O., Ezeonu P.O., Ozonu N.C., Iyoke C.A., Nkwo P.O. and Ajah M.I. (2015) A Five Year Review of Cervical Cytology in Abakaliki, Nigeria. *American Journal of Cancer Prevention* 3, 23-26.

Akarolo-Anthony S.N., Al-Mujtaba M., Famooto A.O., Dareng E.O., Olaniyan O.B., Offiong R., Wheeler C.M. and Adebamowo C.A. (2013) HIV associated high-risk HPV infection among Nigerian women. *BMC infectious diseases* 13, 521.

Allan B., Marais D.J., Hoffman M., Shapiro S. and Williamson A.-L. (2008) Cervical human papillomavirus (HPV) infection in South African women: implications for HPV screening and vaccine strategies. *Journal of clinical microbiology* 46, 740-742.

Ampofo W.K. (2009) Current status of HIV/AIDS treatment, care and support services in Ghana. *Ghana Medical Journal* 43, 142.

Aral S.O. and Holmes K.K. (1999) Social and behavioral determinants of epidemiology of STDs: industrialized and developing countries. . In *Sex Transm Dis*, pp. 39–76 [H.K. K, M.P. A and S.P. F, editors]: New York: McGraw-Hill.

Arbyn M., Sasieni P., Meijer C.J., Clavel C., Koliopoulos G. and Dillner J. (2006a) Chapter 9: Clinical applications of HPV testing: a summary of meta-analyses. *Vaccine* 24 Suppl 3, S3/78-89.

Arbyn M., Sasieni P., Meijer C.J., Clavel C., Koliopoulos G. and Dillner J. (2006b) Clinical applications of HPV testing: a summary of meta-analyses. *Vaccine* 24, S78-S89.

Assoumou S.Z., Mbiguino A.N., Mabika B.M., Ogoula S.N., El Mzibri M., Khattabi A. and Ennaji M.M. (2016) Human papillomavirus genotypes distribution among Gabonese women with normal cytology and cervical abnormalities. *Infectious agents and cancer* 11, 2.

Awofala A.A. and Ogundele O.E. (2018) HIV epidemiology in Nigeria. *Saudi Journal of Biological Sciences* 25, 697-703.

Awuah A. (2014) The Prevalences of Human Papillomavirus Infections and Cervical Lesions, and the Patterns of Risk Behaviour Characteristics of Women in a High Risk Community: Akuse sub-district, Ghana, University of Ghana.

Azzawi M.a. (2013) Investigation of human papilloma viruses infections in prostate cancer, Middlesex University.

Baay M.F., Kjetland E.F., Ndhlovu P.D., Deschoolmeester V., Mduluza T., Gomo E., Friis H., Midzi N., Gwanzura L., Mason P.R., Vermorken J.B. and Gundersen S.G. (2004) Human papillomavirus in a rural community in Zimbabwe: the impact of HIV co-infection on HPV genotype distribution. *J Med Virol* 73, 481-485.

Baseman J.G. and Koutsky L.A. (2005) The epidemiology of human papillomavirus infections. *Journal of clinical virology* 32 Suppl 1, S16-24.

Basnet S., KC S.R. and Geetika K. (2018) Uterine Cervix-Benign Histomorphological Spectrum. *Nepalese Medical Journal* 1, 1-4.

Bedoui S., Whitney P.G., Waithman J., Eidsmo L., Wakim L., Caminschi I., Allan R.S., Wojtasiak M., Shortman K. and Carbone F.R. (2009) Cross-presentation of viral and self antigens by skin-derived CD103+ dendritic cells. *Nature immunology* 10, 488.

Bernard H.-U., Burk R.D., Chen Z., van Doorslaer K., Zur Hausen H. and de Villiers E.-M. (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology* 401, 70-79.

Blossom D., Beigi R., Farrell J., Mackay W., Qadadri B., Brown D., Rwambuya S., Walker C., Kambugu F. and Abdul- Karim F. (2007) Human papillomavirus genotypes associated with cervical cytologic abnormalities and HIV infection in Ugandan women. *J Med Virol* 79, 758-765.

Bosch F., Manos M., Munoz N., Sherman M., Jansen A., Peto J., Schiffman M., Moreno V., Kurman R. and Shah K. (1995a) Prevalence of human Papillomavirus in cervical cancer: a worldwide perspective. *Journal of the National Cancer Institute* 87, 796 - 802.

Bosch F., Munoz N., Shah K. and Meheus A. (1992) Second international workshop on the epidemiology of cervical cancer and human papillomavirus. *International journal of cancer* 52, 171-173.

Bosch F.X., Burchell A.N., Schiffman M., Giuliano A.R., de Sanjose S., Bruni L., Tortolero-Luna G., Kjaer S.K. and Munoz N. (2008) Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. *Vaccine* 26 Suppl 10, K1-16.

Bosch F.X. and de Sanjose S. (2003) Chapter 1: Human papillomavirus and cervical cancer--burden and assessment of causality. *Journal of the National Cancer Institute Monographs*
3-13.

Bosch F.X., Manos M.M., Munoz N., Sherman M., Jansen A.M., Peto J., Schiffman M.H., Moreno V., Kurman R. and Shah K.V. (1995b) Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *Journal of the National Cancer Institute* 87, 796-802.

Bosch F.X., Qiao Y.L. and Castellsagué X. (2006) The epidemiology of human papillomavirus infection and its association with cervical cancer
International Journal of Gynecology and Obstetrics 94, S8-S21.

Boshart M., Gissmann L., Ikenberg H., Kleinheinz A., Scheurlen W. and zur Hausen H. (1984) A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. *The EMBO journal* 3, 1151-1157.

Bousarghin L., Touzé A., Sizaret P.-Y. and Coursaget P. (2003) Human papillomavirus types 16, 31, and 58 use different endocytosis pathways to enter cells. *Journal of virology* 77, 3846-3850.

Brancaccio R.N. (2020) Novel strategies for the identification and full genomic characterization of unknown HPV types from human DNA samples, Université de Lyon.

Bruni L., Diaz M., Castellsagué M., Ferrer E., Bosch F.X. and de Sanjosé S. (2010) Cervical human papillomavirus prevalence in 5 continents: meta-analysis of 1 million women with normal cytological findings. *Journal of Infectious Diseases* 202, 1789-1799.

Burchell A.N., Winer R.L., de Sanjose S. and Franco E.L. (2006) Chapter 6: Epidemiology and transmission dynamics of genital HPV infection. *Vaccine* 24 Suppl 3, S3/52-61.

Burd E. (2003a) Human papillomavirus and cervical cancer. *Clinical Microbiology Reviews* 16, 1 - 17.

Burd E.M. (2003b) Human papillomavirus and cervical cancer. *Clinical Microbiology Reviews* 16, 1-17.

Burk R.D., Chen Z. and Van Doorslaer K. (2009) Human papillomaviruses: genetic basis of carcinogenicity. *Public health genomics* 12, 281-290.

Castellsague X., Menendez C., Loscertales M.P., Kornegay J.R., dos Santos F., Gomez-Olive F.X., Lloveras B., Abarca N., Vaz N., Barreto A., Bosch F.X. and Alonso P. (2001) Human papillomavirus genotypes in rural Mozambique. *The Lancet* 358, 1429-1430.

Castle P.E., Rodríguez A.C., Burk R.D., Herrero R., Wacholder S., Alfaro M., Morales J., Guillen D., Sherman M.E. and Solomon D. (2009) Short term persistence of human papillomavirus and risk of cervical precancer and cancer: population based cohort study. *BMJ* 339.

Chan K.M.M., Cheng Fung Yip M., Gwi E. and Chan Hei Tung T. (2002) Cervical Cytology Practice Guidelines [C.C.P.G. Group, editor. Hong Kong: Hong Kong Society of Cytology

Chaturvedi A.K. (2010) Beyond cervical cancer: burden of other HPV-related cancers among men and women. *Journal of Adolescent Health* 46, S20-S26.

Chaturvedi A.K., Dumestre J., Gaffga A.M., Mire K.M., Clark R.A., Braly P.S., Dunlap K., Beckel T.E., Hammons A.F., Kissinger P.J. and Hagensee M.E. (2005) Prevalence of human papillomavirus genotypes in women from three clinical settings. *J Med Virol* 75, 105-113.

Chaturvedi A.K., Madeleine M.M., Biggar R.J. and Engels E.A. (2009) Risk of human papillomavirus–associated cancers among persons with AIDS. *JNCI: Journal of the National Cancer Institute* 101, 1120-1130.

Chuang T.-Y. (1987) Condylomata acuminata (genital warts): an epidemiologic view. *Journal of the American Academy of Dermatology* 16, 376-384.

Clifford G., Franceschi S., Diaz M., Munoz N. and Villa L.L. (2006a) Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine* 24 Suppl 3, S3/26-34.

Clifford G., Franceschi S., Diaz M., Munoz N. and Villa L.L. (2006b) HPV type-distribution in women with and without cervical neoplastic diseases. *Vaccine* 24, S26-S34.

Clifford G., Gallus S., Herrero R., Munoz N., Snijders P., Vaccarella S., Anh P., Ferreccio C., Hieu N. and Matos E. (2005a) Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *The Lancet* 366, 991-998.

Clifford G., Smith J., Plummer M., Munoz N. and Franceschi S. (2003) Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *British Journal of Cancer* 88, 63.

Clifford G.M., Gallus S., Herrero R., Munoz N., Snijders P.J., Vaccarella S., Anh P.T., Ferreccio C., Hieu N.T., Matos E., Molano M., Rajkumar R., Ronco G., de Sanjose S., Shin H.R., Sukvirach S., Thomas J.O., Tunsakul S., Meijer C.J. and Franceschi S. (2005b) Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: a pooled analysis. *The Lancet* 366, 991-998.

Clifford G.M., Goncalves M.A.G. and Franceschi S. (2006c) Human papillomavirus types among women infected with HIV: a meta-analysis. *Aids* 20, 2337-2344.

Clifford G.M., Goncalves M.A.G., Franceschi S., HPV and group H.s. (2006d) Human papillomavirus types among women infected with HIV: a meta-analysis. *Aids* 20, 2337-2344.

Clifford G.M., Tully S. and Franceschi S. (2017) Carcinogenicity of human papillomavirus (HPV) types in HIV-positive women: a meta-analysis from HPV infection to cervical cancer. *Clinical infectious diseases* 64, 1228-1235.

Cogliano V., Baan R., Straif K., Grosse Y., Secretan B. and El Ghissassi F. (2005) Carcinogenicity of human papillomaviruses. *The lancet oncology* 6, 204.

Collins S., Mazloomzadeh S., Winter H., Blomfield P., Bailey A., Young L.S. and Woodman C.B. (2002) High incidence of cervical human papillomavirus infection in women during their first sexual relationship. *BJOG* 109, 96-98.

Cosper P.F., Bradley S., Luo Q. and Kimple R.J. (2021) Biology of HPV Mediated Carcinogenesis and Tumor Progression. *Seminars in Radiation Oncology* **31**, 265-273.

Coutlee F., Gravitt P., Kornegay J., Hankins C., Richardson H., Lapointe N., Voyer H. and Franco E. (2002) Use of PGMY primers in L1 consensus PCR improves detection of human papillomavirus DNA in genital samples. *Journal of Clinical Microbiology*
40, 902-907.

Crosbie E.J., Einstein M.H., Franceschi S. and Kitchener H.C. (2013) Human papillomavirus and cervical cancer. *The Lancet* 382, 889-899.

Cullen M., Boland J.F., Schiffman M., Zhang X., Wentzensen N., Yang Q., Chen Z., Yu K., Mitchell J. and Roberson D. (2015) Deep sequencing of HPV16 genomes: A new high-throughput tool for exploring the carcinogenicity and natural history of HPV16 infection. *Papillomavirus research* 1, 3-11.

Curtis S.L. and Sutherland E.G. (2004) Measuring sexual behaviour in the era of HIV/AIDS: the experience of Demographic and Health Surveys and similar enquiries. *Sex Transm Infect* 80 Suppl 2, ii22-27.

Cuschieri K.S., Whitley M.J. and Cubie H.A. (2004) Human papillomavirus type specific DNA and RNA persistence--implications for cervical disease progression and monitoring. *J Med Virol* 73, 65-70.

Cutts F.T., Franceschi S., Goldie S., Castellsague X., de Sanjose S., Garnett G., Edmunds W.J., Claeys P., Goldenthal K.L., Harper D.M. and Markowitz L. (2007) Human papillomavirus and HPV vaccines: a review. *Bull World Health Organ* 85, 719-726.

Cuzick J., Szarewski A., Cubie H., Hulman G., Kitchener H., Luesley D., McGoogan E., Menon U., Terry G. and Edwards R. (2003) Management of women who test positive for high-risk types of human papillomavirus: the HART study. *The Lancet* 362, 1871-1876.

Dalstein V., Riethmuller D., Prétet J.L., Le Bail Carval K., Sautière J.L., Carbillet J.P., Kantelip B., Schaal J.P. and Mougin C. (2003) Persistence and load of high- risk HPV are predictors for development of high- grade cervical lesions: a longitudinal French cohort study. *International journal of cancer* 106, 396-403.

Dames D.N., Ragin C., Griffith-Bowe A., Gomez P. and Butler R. (2009) The prevalence of cervical cytology abnormalities and human papillomavirus in women infected with the human immunodeficiency virus. *Infectious agents and cancer* 4, S8.

Davey D.D. (2005) Cytopathology update on atypical squamous cells. *Journal of lower genital tract disease* 9, 124-129.

De Lemos P., García-Zapata M. and Tavares S. (2012) Cervical cytopathology in a population of HIV-positive and HIV-negative women. *Journal of tropical medicine* 2012.

de Sanjose S., Almirall R., Lloveras B., Font R., Diaz M., Munoz N., Catala I., Meijer C.J., Snijders P.J., Herrero R. and Bosch F.X. (2003) Cervical human papillomavirus infection in the female population in Barcelona, Spain. *Sexually Transmitted Diseases*
30, 788-793.

de Sanjose S., Brotons M. and Pavon M.A. (2018) The natural history of human papillomavirus infection. *Best practice & research Clinical obstetrics & gynaecology* 47, 2-13.

de Sanjose S., Diaz M., Castellsague X., Clifford G., Bruni L., Munoz N. and Bosch F.X. (2007) Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *The Lancet Infect Dis* 7, 453-459.

De Sanjosé S., Diaz M., Castellsagué X., Clifford G., Bruni L., Muñoz N. and Bosch F.X. (2007) Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis* 7, 453-459.

de Sanjosé S., Pérez C., Velasco J., Klaustermeier J., Alemany L., Tous S., Quint W. and Bosch F. (2011) Vaccine-related HPV Genotypes in Invasive Cervical Cancer in Asturias, Spain. *27th INTERNATIONAL PAPILLOMAVIRUS CONFERENCE AND CLINICAL WORKSHOP*, 06:24.

de Sanjose S., Quint W.G., Alemany L., Geraets D.T., Klaustermeier J.E., Lloveras B., Tous S., Felix A., Bravo L.E. and Shin H.-R. (2010) Human papillomavirus genotype attribution in invasive cervical cancer: a retrospective cross-sectional worldwide study. *The lancet oncology* 11, 1048-1056.

de Villiers E.-M. (2013) Cross-roads in the classification of papillomaviruses. *Virology* 445, 2-10.

De Villiers E.-M., Fauquet C., Broker T.R., Bernard H.-U. and zur Hausen H. (2004) Classification of papillomaviruses. *Virology* 324, 17-27.

De Vuyst H., Clifford G.M., Nascimento M.C., Madeleine M.M. and Franceschi S. (2009) Prevalence and type distribution of human papillomavirus in carcinoma and intraepithelial neoplasia of the vulva, vagina and anus: a meta- analysis. *International journal of cancer* 124, 1626-1636.

De Vuyst H., Steyaert S., Van Renterghem L., Claeys P., Muchiri L., Sitati S., Vansteelandt S., Quint W., Kleter B., Van Marck E. and Temmerman M. (2003) Distribution of human papillomavirus in a family planning population in nairobi, kenya. *Sex Transm Dis* 30, 137-142.

Denny L.A., Franceschi S., de Sanjosé S., Heard I., Moscicki A.B. and Palefsky J. (2012) Human papillomavirus, human immunodeficiency virus and immunosuppression. *Vaccine* 30, F168-F174.

Desruisseau A.J., Schmidt-Grimminger D. and Welty E. (2009) Epidemiology of HPV in HIV-positive and HIV-negative fertile women in Cameroon, West Africa. *Infect Dis Obstetrics and Gynecology* 2009, 810596.

Dhamija S. and Menon M.B. (2018) Non-coding transcript variants of protein-coding genes–what are they good for? *RNA biology* 15, 1025-1031.

Didelot-Rousseau M.-N., Nagot N., Costes-Martineau V., Valles X., Ouedraogo A., Konate I., Weiss H.A., Van de Perre P., Mayaud P. and Segondy M. (2006) Human papillomavirus genotype distribution and cervical squamous intraepithelial lesions among high-risk women with and without HIV-1 infection in Burkina Faso. *British Journal of Cancer* 95, 355-362.

DiMaio D. and Petti L.M. (2013) The E5 proteins. *Virology* 445, 99-114.

Domfeh A., Wiredu E., Adjei A., Ayeh-Kumi P., Adiku T., Tettey Y., Gyasi R. and Armah H. (2008) Cervical human papillomavirus infection in accra, ghana. *Ghana Medical Journal* 42, 71-78.

Donkoh E. (2015) Distribution of Genital Human Papillomaviruses and Associated Cervical Disease in Kumasi, Kwame Nkrumah University of Science and Technology.

Donkoh E.T. (2017) Distribution of Human Papillomavirus (HPV) genotypes and associated cervical disease in an unscreened population of women in Kumasi, Ghana.

Donkoh E.T., Agyemang-Yeboah F., Asmah R.H. and Wiredu E.K. (2019a) Prevalence of cervical cancer and pre-cancerous lesions among unscreened Women in Kumasi, Ghana. *Medicine* 98, e14600.

Donkoh E.T., Agyemang-Yeboah F., Asmah R.H. and Wiredu E.K. (2019b) Prevalence of cervical cancer and pre-cancerous lesions among unscreened Women in Kumasi, Ghana. *Medicine* 98.

Doorbar J. (2013) The E4 protein; structure, function and patterns of expression. *Virology* 445, 80-98.

Doorbar J., Quint W., Banks L., Bravo I.G., Stoler M., Broker T.R. and Stanley M.A. (2012) The biology and life-cycle of human papillomaviruses. *Vaccine* 30, F55-F70.

Duru C., Oluoha R., Uwakwe K., Diwe K., Merenu I., Emerole C., Ndukwu E. and Iwu C. (2015) Pattern of Pap Smear Test Result among Nigerian women attending Clinics in a Teaching Hospital. *Int. J. Curr. Microbiol. App. Sci* 4, 986-998.

Fang L., Lin X., Yang Y., Song Z., Ding X., Tan L. and Gao P. (2020) Genetic variability, phylogeny and functional implication of the long control region in human papillomavirus type 16, 18 and 58 in Chengdu, China. *Virology Journal* 17, 1-15.

Ferenczy A., Coutlee F., Franco E. and Hankins C. (2003) Human papillomavirus and HIV coinfection and the risk of neoplasias of the lower genital tract: a review of recent developments. *CMAJ* 169, 431-434.

Ferenczy A., Gelfand M.M., Franco E. and Mansour N. (1997) Human papillomavirus infection in postmenopausal women with and without hormone therapy. *Obstetrics and Gynecology* 90, 7-11.

Fleury M.J., Touze A., de Sanjose S., Bosch F.X., Klaustermeiyer J. and Coursaget P. (2008) Detection of human papillomavirus type 31-neutralizing antibodies from naturally infected patients by an assay based on intracellular assembly of luciferase-expressing pseudovirions. *Clin Vaccine Immunol* 15, 172-175.

Fonn S., Bloch B., Mabina M., Carpenter S., Cronje H., Maise C., Bennun M., Du Toit G., De Jonge E. and Manana I. (2015) Prevalence of pre-cancerous lesions and cervical cancer in South Africa-a multicentre study. *South African Medical Journal* 92, 148-156.

Forman D., de Martel C., Lacey C.J., Soerjomataram I., Lortet-Tieulent J., Bruni L., Vignat J., Ferlay J., Bray F., Plummer M. and Franceschi S. (2012) Global burden of human papillomavirus and related diseases. *Vaccine* 30 Suppl 5, F12-23.

Forslund O., Lindelöf B., Hradil E., Nordin P., Stenquist B., Kirnbauer R., Slupetzky K. and Dillner J. (2004) High prevalence of cutaneous human papillomavirus DNA on the top of skin tumors but not in "Stripped" biopsies from the same tumors. *Journal of Investigative Dermatology* 123, 388-394.

Franceschi S., Herrero R., Clifford G.M., Snijders P.J., Arslan A., Anh P.T.H., Bosch F.X., Ferreccio C., Hieu N.T. and Lazcano- Ponce E. (2006) Variations in the age- specific curves of human papillomavirus prevalence in women worldwide. *International journal of cancer* 119, 2677-2684.

Franceschi S. and Jaffe H. (2007) Cervical cancer screening of women living with HIV infection: a must in the era of antiretroviral therapy. *Clinical infectious diseases* 45, 510-513.

Franco E.L., Cuzick J., Hildesheim A. and de Sanjose S. (2006) Chapter 20: Issues in planning cervical cancer screening in the era of HPV vaccination. *Vaccine* 24 Suppl 3, S3/171-177.

Frellick M. (2017) Updated guideline on cervical cancer screening issued by ACOG. Medscape. December 24, 2015.

Frisch M., Biggar R.J. and Goedert J.J. (2000) Human papillomavirus-associated cancers in patients with human immunodeficiency virus infection and acquired immunodeficiency syndrome. *Journal of the National Cancer Institute* 92, 1500-1510.

Garutti P., Montori S., Bazzan E. and Tarabbia C. (2018) Gender differences in the epidemiology and prevention of human papillomavirus (HPV) and HPV-related diseases. *Ital. J. Gend.-Specif. Med* 4, 152-156.

Gasparini R. and Panatto D. (2009) Cervical cancer: from Hippocrates through Rigoni-Stern to zur Hausen.

Gelband H. and Sloan F.A. (2007) *Cancer control opportunities in low-and middle-income countries*: National Academies Press.

Gellin J., Brown S., Marshall Graves J.A., Rothschild M., Schook L., Womack J. and Yerle M. (2000) Comparative gene mapping workshop: progress in agriculturally important animals. *Mamm Genome* 11, 140-144.

Georgescu S.R., Mitran C.I., Mitran M.I., Caruntu C., Sarbu M.I., Matei C., Nicolae I., Tocut S.M. and Popa M.I. (2018) New insights in the pathogenesis of HPV infection and the associated carcinogenic processes: the role of chronic inflammation and oxidative stress. *Journal of immunology research* 2018.

Getinet M., Gelaw B., Sisay A., Mahmoud E.A. and Assefa A. (2015) Prevalence and predictors of Pap smear cervical epithelial cell abnormality among HIV-positive and negative women attending gynecological examination in cervical cancer screening center at Debre Markos referral hospital, East Gojjam, Northwest Ethiopia. *BMC clinical pathology* 15, 1.

Ghana A. (2012) Commission: Ghana's Progress Report on the United Nations General Assembly Special Session (UNGASS) Declaration of Commitment on HIV and AIDS. Ghana AIDS Commission and UNAIDS. 2010.

Ghebre R.G., Grover S., Xu M.J., Chuang L.T. and Simonds H. (2017) Cervical cancer control in HIV-infected women: past, present and future. *Gynecologic oncology reports* 21, 101-108.

Gheit T. (2019) Mucosal and cutaneous human papillomavirus infections and cancer biology. *Frontiers in Oncology* 9, 355.

Ginsburg O., Bray F., Coleman M.P., Vanderpuye V., Eniu A., Kotha S.R., Sarker M., Huong T.T., Allemani C. and Dvaladze A. (2017) The global burden of women's cancers: a grand challenge in global health. *The Lancet* 389, 847-860.

Gjooen K., Olsen A.O., Magnus P., Grinde B., Sauer T. and Orstavik I. (1996) Prevalence of human papillomavirus in cervical scrapes, as analyzed by PCR, in a population-based sample of women with and without cervical dysplasia. *APMIS* 104, 68-74.

Goncalves M.A., Massad E., Burattini M.N. and Villa L.L. (1999) Relationship between human papillomavirus (HPV) genotyping and genital neoplasia in HIV-positive patients of Santos City, Sao Paulo, Brazil. *Int J STD AIDS* 10, 803-807.

Gondos A., Brenner H., Wabinga H. and Parkin D. (2005) Cancer survival in Kampala, Uganda. *British Journal of Cancer* 92, 1808.

Gravitt P.E., Kamath A.M., Gaffikin L., Chirenje Z.M., Womack S. and Shah K.V. (2002) Human papillomavirus genotype prevalence in high-grade squamous intraepithelial lesions and colposcopically normal women from Zimbabwe. *International journal of cancer* 100, 729-732.

Guimarães M.D.C., Grinsztejn B., Melo V.H., Rocha G.M., Campos L.N., Pilotto J.H., Carmo R.A. and Palefsky J.M. (2011) Anal HPV prevalence and associated factors among HIV-seropositive men under antiretroviral treatment in Brazil. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 57, S217-S224.

Handlogten K.S., Molitor R.J., Roeker L.E., Narla N.P., Bachman M.J., Quayson S., Owusu-Afriyie O., Adjei E., Ankobea F. and Clayton A. (2014) Cervical Cancer Screening in Ghana, West Africa: Prevalence of Abnormal Cytology and Challenges for Expanding Screening. *International Journal of Gynecologic Pathology* 33, 197-202.

Harms T., Cryer D. and Clifford R.M. (2006) *Infant/toddler environment rating scale*: Teachers College Press New York.

Harper D.M., Franco E.L., Wheeler C., Ferris D.G., Jenkins D., Schuind A., Zahaf T., Innis B., Naud P., De Carvalho N.S., Roteli-Martins C.M., Teixeira J., Blatter M.M., Korn A.P., Quint W. and Dubin G. (2004) Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *The Lancet* 364, 1757-1765.

Hawes S.E., Critchlow C.W., Sow P.S., Touré P., N'Doye I., Diop A., Kuypers J.M., Kasse A.A. and Kiviat N.B. (2006) Incident high-grade squamous intraepithelial lesions in Senegalese women with and without human immunodeficiency virus type 1 (HIV-1) and HIV-2. *Journal of the National Cancer Institute* 98, 100-109.

Herrero R., Castellsagué X., Pawlita M., Lissowska J., Kee F., Balaram P., Rajkumar T., Sridhar H., Rose B. and Pintos J. (2003) Human papillomavirus and oral cancer: the International Agency for Research on Cancer multicenter study. *Journal of the National Cancer Institute* 95, 1772-1783.

Herrero R., Castle P.E., Schiffman M., Bratti M.C., Hildesheim A., Morales J., Alfaro M., Sherman M.E., Wacholder S., Chen S., Rodriguez A.C. and Burk R.D. (2005) Epidemiologic profile of type-specific human papillomavirus infection and cervical neoplasia in Guanacaste, Costa Rica. *Journal of Infectious Diseases* 191, 1796-1807.

HIV U.G. AIDS statistics—2018 fact sheet.

HIV/AIDS J.U.N.P.o. (2018) Global Aids Update 2016. Geneva: UNAIDS, 2016.

Ho G.Y., Burk R.D., Klein S., Kadish A.S., Chang C.J., Palan P., Basu J., Tachezy R., Lewis R. and Romney S. (1995) Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *Journal of the National Cancer Institute* 87, 1365-1371.

Hording U., Daugaard S., Junge J. and Lundvall F. (1996) Human papillomaviruses and multifocal genital neoplasia. *International journal of gynecological pathology: official journal of the International Society of Gynecological Pathologists* 15, 230-234.

Humans I.W.G.o.t.E.o.C.R.t., Meeting I.W.G.o.t.E.o.C.R.t.H., Organization W.H. and Cancer I.A.f.R.o. (2007) *Human papillomaviruses*: World Health Organization.

IARC (1995) Epidemiology of infection: human papillomaviruses *Carcinog Risk Chem Hum* 64, 60-65.

IARC and WHO d. (2008) Specific methodology for Ghana: Simple mean of estimate for Cote d'Ivoire, Niger and Burkina Faso. In *GLOBOCAN 2008*. Lyon, France: IARC, WHO.

Ina S., Lucia R., Carolina C. and Laura A. (2021) Human Papillomavirus and Its Role in the Development of Cancer. In *Human Viruses: Diseases, Treatments and Vaccines*, pp. 299-310: Springer.

Isaakidis P., Pimple S., Varghese B., Khan S., Mansoor H., Ladomirska J., Sharma N., Silva E.D., Metcalf C. and Caluwaerts S. (2013) HPV infection, cervical abnormalities, and cancer in HIV-infected women in Mumbai, India: 12-month follow-up. *International journal of women's health*.

Jamieson D.J., Duerr A., Burk R., Klein R.S., Paramsothy P., Schuman P., Cu-Uvin S., Shah K. and Group H.E.R.S. (2002) Characterization of genital human papillomavirus infection in women who have or who are at risk of having HIV infection. *Am J Obstet Gynecol* 186, 21-27.

Jenkins D. (2007) Histopathology and cytopathology of cervical cancer. *Disease markers* 23, 199-212.

Jeon S., Allen-Hoffmann B.L. and Lambert P.F. (1995) Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. *Journal of virology* 69, 2989-2997.

Kayumba P. (2014) Prevalence of Cervical Cytology Abnormalities Among HIV Infected Women At Rwanda Military Hospital: A Cross-Sectional Desriptive Study, University of Nairobi.

Keita N., Clifford G., Koulibaly M., Douno K., Kabba I., Haba M., Sylla B., Van Kemenade F., Snijders P. and Meijer C. (2009) HPV infection in women with and without cervical cancer in Conakry, Guinea. *British Journal of Cancer* 101, 202-208.

Khan M.J., Castle P.E., Lorincz A.T., Wacholder S., Sherman M., Scott D.R., Rush B.B., Glass A.G. and Schiffman M. (2005) The elevated 10-year risk of cervical precancer and cancer in women with human papillomavirus (HPV) type 16 or 18 and the possible utility of type-specific HPV testing in clinical practice. *Journal of the National Cancer Institute* 97, 1072-1079.

Kjaer S.K., Chackerian B., van den Brule A.J., Svare E.I., Paull G., Walbomers J.M., Schiller J.T., Bock J.E., Sherman M.E., Lowy D.R. and Meijer C.L. (2001) High-risk human papillomavirus is sexually transmitted: evidence from a follow-up study of virgins starting sexual activity (intercourse). *Cancer Epidemiology Biomarkers & Prevention* 10, 101-106.

Kjaer S.K., van den Brule A.J.C., Bock J.E., Poll P.A., Engholm G., Sherman M.E., Walboomers J.M.M. and Meijer C. (1996) Human papillomavirus-The most significant risk determinant of cervical intraepithelial neoplasia. *International journal of cancer* 65, 601-606.

Koch A., Hansen S.V., Nielsen N.M., Palefsky J. and Melbye M. (1997) HPV detection in children prior to sexual debut. *International journal of cancer* 73, 621-624.

Koss L.G. and Melamed M.R. (2006) *Koss' diagnostic cytology and its histopathologic bases*: Lippincott Williams & Wilkins.

Koutsky L.A. and Harper D.M. (2006) Current findings from prophylactic HPV vaccine trials. *Vaccine* 24, S114-S121.

Kranjec C., Tomaić V., Massimi P., Nicolaides L., Doorbar J. and Banks L. (2016) The high-risk HPV E6 target scribble (hScrib) is required for HPV E6 expression in cervical tumour-derived cell lines. *Papillomavirus Research* 2, 70-77.

Kriek J.-M., Jaumdally S.Z., Masson L., Little F., Mbulawa Z., Gumbi P.P., Barnabas S.L., Moodley J., Denny L. and Coetzee D. (2016) Female genital tract inflammation, HIV co-infection and persistent mucosal Human Papillomavirus (HPV) infections. *Virology* 493, 247-254.

Lacey C.J., Lowndes C.M. and Shah K.V. (2006) Chapter 4: Burden and management of non-cancerous HPV-related conditions: HPV-6/11 disease. *Vaccine* 24 Suppl 3, S3/35-41.

Laryea D.O., Awuah B., Amoako Y.A., Osei-Bonsu E., Dogbe J., Larsen-Reindorf R., Ansong D., Yeboah-Awudzi K., Oppong J.K. and Konney T.O. (2014) Cancer incidence in Ghana, 2012: evidence from a population-based cancer registry. *BMC cancer* 14, 362.

Lazcano-Ponce E., Herrero R., Munoz N., Cruz A., Shah K.V., Alonso P., Hernandez P., Salmeron J. and Hernandez M. (2001) Epidemiology of HPV infection among Mexican women with normal cervical cytology. *International journal of cancer* 91, 412-420.

Lehtovirta P., Paavonen J. and Heikinheimo O. (2008) Risk factors, diagnosis and prognosis of cervical intraepithelial neoplasia among HIV-infected women. *Int J STD AIDS* 19, 37-41.

Ley C., Bauer H.M., Reingold A., Schiffman M.H., Chambers J.C., Tashiro C.J. and Manos M.M. (1991) Determinants of genital human papillomavirus infection in young women. *Journal of the National Cancer Institute* 83, 997-1003.

Liaw K.L., Glass A.G., Manos M.M., Greer C.E., Scott D.R., Sherman M., Burk R.D., Kurman R.J., Wacholder S., Rush B.B., Cadell D.M., Lawler P., Tabor D. and Schiffman M. (1999) Detection of human papillomavirus DNA in cytologically normal women and subsequent cervical squamous intraepithelial lesions. *Journal of the National Cancer Institute* 91, 954-960.

Liaw K.L., Hildesheim A., Burk R.D., Gravitt P., Wacholder S., Manos M.M., Scott D.R., Sherman M.E., Kurman R.J., Glass A.G., Anderson S.M. and Schiffman M. (2001) A prospective study of human papillomavirus (HPV) type 16 DNA detection by polymerase chain reaction and its association with acquisition and persistence of other HPV types. *Journal of Infectious Diseases* 183, 8-15.

Lörincz A.T. and Richart R.M. (2003) Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programs. *Archives of pathology & laboratory medicine* 127, 959-968.

Lu B., Kumar A., Castellsagué X. and Giuliano A.R. (2011) Efficacy and safety of prophylactic vaccines against cervical HPV infection and diseases among women: a systematic review & meta-analysis. *BMC infectious diseases* 11, 13.

Luchters S.M., Broeck D.V., Chersich M.F., Nel A., Delva W., Mandaliya K., Depuydt C.E., Claeys P., Bogers J.-P. and Temmerman M. (2010) Association of HIV infection with distribution and viral load of HPV types in Kenya: a survey with 820 female sex workers. *BMC infectious diseases* 10, 18.

Manhart L.E. and Koutsky L.A. (2002) Do condoms prevent genital HPV infection, external genital warts, or cervical neoplasia? A meta-analysis. *Sexually Transmitted Diseases* 29, 725-735.

Marie-Eve R., Petros I., Chutema P. and Tony R. (2009) Screening and treating cervical cancer in HIV-positive women in Cambodia. *JAIDS Journal of Acquired Immune Deficiency Syndromes* 51, 644-646.

Massad L.S., Xie X., Burk R.D., D'Souza G., Darragh T.M., Minkoff H., Colie C., Burian P., Palefsky J. and Atrio J. (2016) Association of cervical precancer with human papillomavirus types other than 16 among HIV co-infected women. *Am J Obstet Gynecol* 214, 354. e351-354. e356.

Mbulaiteye S.M., Bhatia K., Adebamowo C. and Sasco A.J. (2011) HIV and cancer in Africa: mutual collaboration between HIV and cancer programs may provide timely research and public health data. *Infectious agents and cancer* 6, 16.

Mbulaiteye S.M., Katabira E.T., Wabinga H., Parkin D.M., Virgo P., Ochai R., Workneh M., Coutinho A. and Engels E.A. (2006) Spectrum of cancers among HIV- infected persons in Africa: the Uganda AIDS-Cancer Registry Match Study. *International journal of cancer* 118, 985-990.

McBride A.A. (2017) Oncogenic human papillomaviruses. *Philosophical Transactions of the Royal Society B: Biological Sciences* 372, 20160273.

McBride A.A. (2021) Human papillomaviruses: Diversity, infection and host interactions. *Nature Reviews Microbiology*, 1-14.

McDonald A.C., Tergas A.I., Kuhn L., Denny L. and Wright T.C. (2014) Distribution of human papillomavirus genotypes among HIV-positive and HIV-negative women in Cape Town, South Africa. *Frontiers in Oncology* 4, 48.

McKee P., Wilkinson J., Corbett M., Davey A., Sauven P. and Black M. (1981) Carcinoma cuniculatum: a case metastasizing to skin and lymph nodes. *Clinical and experimental dermatology* 6, 613-618.

Mehanna H., Beech T., Nicholson T., El- Hariry I., McConkey C., Paleri V. and Roberts S. (2013) Prevalence of human papillomavirus in oropharyngeal and nonoropharyngeal head and neck cancer—systematic review and meta- analysis of trends by time and region. *Head & neck* 35, 747-755.

Meloni A., Pilia R., Campagna M., Usai A., Masia G., Caredda V. and Coppola R.C. (2014) Prevalence and molecular epidemiology of human papillomavirus infection in Italian women with cervical cytological abnormalities. *Journal of public health research* 3.

Memiah P., Mbuthia W., Kiiru G., Agbor S., Odhiambo F., Ojoo S. and Biadgilign S. (2012) Prevalence and risk factors associated with precancerous cervical cancer lesions among HIV-infected women in resource-limited settings. *AIDS research and treatment* 2012.

Mendez F., Munoz N., Posso H., Molano M., Moreno V., van den Brule A.J., Ronderos M., Meijer C. and Munoz A. (2005) Cervical coinfection with human papillomavirus (HPV) types and possible implications for the prevention of cervical cancer by HPV vaccines. *Journal of Infectious Diseases* 192, 1158-1165.

Menon S., Wusiman A., Boily M.C., Kariisa M., Mabeya H., Luchters S., Forland F., Rossi R., Callens S. and vanden Broeck D. (2016) Epidemiology of HPV Genotypes among HIV Positive Women in Kenya: A Systematic Review and Meta-Analysis. *PLOS ONE* 11, e0163965.

Miller W.B. and Torday J.S. (2017) A systematic approach to cancer: evolution beyond selection. *Clinical and translational medicine* 6, 2.

Minkoff H., Feldman J., DeHovitz J., Landesman S. and Burk R. (1998) A longitudinal study of human papillomavirus carriage in human immunodeficiency virus-infected and human immunodeficiency virus-uninfected women. *Am J Obstet Gynecol* 178, 982-986.

Minkoff H., Zhong Y., Burk R.D., Palefsky J.M., Xue X., Watts D.H., Levine A.M., Wright R.L., Colie C. and D'Souza G. (2010) Influence of Adherent and Effective Antiretroviral Therapy Use on Human Papillomavirus Infection and Squamous Intraepithelial Lesions in Human Immunodeficiency Virus—Positive Women. *The Journal of Infectious Diseases* 201, 681-690.

Molano M., Van den Brule A., Plummer M., Weiderpass E., Posso H., Arslan A., Meijer C.J., Munoz N. and Franceschi S. (2003) Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. *Am J Epidemiol* 158, 486-494.

Moodley M. and Mould S. (2005) Invasive cervical cancer and human immunodeficiency virus (HIV) infection in KwaZulu-Natal, South Africa. *Journal of obstetrics and gynaecology* 25, 706-710.

Moscicki A.B., Ellenberg J.H., Farhat S. and Xu J. (2004) Persistence of human papillomavirus infection in HIV-infected and -uninfected adolescent girls: risk factors and differences, by phylogenetic type. *Journal of Infectious Diseases* 190, 37-45.

Münger K. and Howley P.M. (2002) Human papillomavirus immortalization and transformation functions. *Virus research* 89, 213-228.

Münger K., Scheffner M., Huibregtse J. and Howley P. (1992) Interactions of HPV E6 and E7 oncoproteins with tumour suppressor gene products. *Cancer surveys* 12, 197-217.

Munoz N., Bosch F., de Sanjose S., Herrero R., Castellsague X., Shah K., Snijders P., Meijer C. and Group I.A.f.R.o.C.M.C.C.S. (2003a) Epidemiologic classification of human papillomavirus types associated with cervical cancer. *New England Journal of Medicine* 348, 518 - 527.

Munoz N., Bosch F.X., Castellsague X., Diaz M., de Sanjose S., Hammouda D., Shah K.V. and Meijer C.J. (2004) Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *International journal of cancer* 111, 278-285.

Munoz N., Bosch F.X., de Sanjose S., Herrero R., Castellsague X., Shah K.V., Snijders P.J. and Meijer C.J. (2003b) Epidemiologic classification of human papillomavirus types associated with cervical cancer. *New England Journal of Medicine* 348, 518-527.

Muñoz N., Bosch F.X., de Sanjosé S., Herrero R., Castellsagué X., Shah K.V., Snijders P.J. and Meijer C.J. (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. *New England Journal of Medicine* 348, 518-527.

Munoz N., Kato I., Bosch F.X., Eluf-Neto J., De Sanjose S., Ascunce N., Gili M., Izarzugaza I., Viladiu P., Tormo M.J., Moreo P., Gonzalez L.C., Tafur L., Walboomers J.M. and Shah K.V. (1996) Risk factors for HPV DNA detection in middle-aged women. *Sex Transm Dis* 23, 504-510.

Muñoz N., Méndez F., Posso H., Molano M., Van Den Brule A.J., Ronderos M., Meijer C., Muñoz Á. and Group I.N.d.C.H.S. (2004) Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. *The Journal of Infectious Diseases* 190, 2077-2087.

Muzyka A., Tarkany O., Novikov A., Moskalenko E., Vysotsky A. and Volokh V. (2007) GraphPad Prism [D. Radushev, editor. San Diego, California: GraphPad Software, Inc.

Nicolàs Pàrraga S. (2018) Genetic diversity of human papillomavirus infections in anogenital warts and cancers.

Nobbenhuis M.A.E., Walboomers J.M.M., Helmerhorst T.J.M., Rozendaal L., Remmink A.J., Risse E.K.J., van der Linden H.C., Voorhorst F.J., Kenemans P. and Meijer C.J.L. (1999) Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. *The Lancet* 354, 20-25.

Nobre R.J., Herráez-Hernández E., Fei J.-W., Langbein L., Kaden S., Gröne H.-J. and de Villiers E.-M. (2009) E7 oncoprotein of novel human papillomavirus type 108 lacking the E6 gene induces dysplasia in organotypic keratinocyte cultures. *Journal of virology* 83, 2907-2916.

Obiri-Yeboah D., Akakpo P.K., Mutocheluh M., Adjei-Danso E., Allornuvor G., Amoako-Sakyi D., Adu-Sarkodie Y. and Mayaud P. (2017) Epidemiology of cervical human papillomavirus (HPV) infection and squamous intraepithelial lesions (SIL) among a cohort of HIV-infected and uninfected Ghanaian women. *BMC cancer* 17, 688.

Odida M., Sandin S., Mirembe F., Kleter B., Quint W. and Weiderpass E. (2011) HPV types, HIV and invasive cervical carcinoma risk in Kampala, Uganda: a case-control study. *Infectious agents and cancer* 6, 8.

Organization W.H. and Cancer I.A.f.R.o. (2007) *Human papillomaviruses*: World Health Organization.

Palefsky J.M., Minkoff H., Kalish L.A., Levine A., Sacks H.S., Garcia P., Young M., Melnick S., Miotti P. and Burk R. (1999) Cervicovaginal human papillomavirus infection in human immunodeficiency virus-1 (HIV)-positive and high-risk HIV-negative women. *Journal of the National Cancer Institute* 91, 226-236.

Park E.K., Cho H., Lee S.H., Lee S.G., Lee S.Y., Kim K.H., Lee C.H., Chung J.S. and Kwak I.S. (2014) Human papillomavirus prevalence and genotype distribution among HIV-infected women in Korea. *Journal of Korean medical science* 29, 32-37.

Park L.S., Hernández-Ramírez R.U., Silverberg M.J., Crothers K. and Dubrow R. (2016) Prevalence of non-HIV cancer risk factors in persons living with HIV/AIDS: a meta-analysis. *AIDS (London, England)* 30, 273.

Park T.W., Fujiwara H. and Wright T.C. (1995) Molecular biology of cervical cancer and its precursors. *Cancer* 76, 1902-1913.

Parkin D.M. and Bray F. (2006) Chapter 2: The burden of HPV-related cancers. *Vaccine* 24 Suppl 3, S3/11-25.

Parkin D.M., Ferlay J., Hamdi-Cherif M., Sitas F., Thomas J.O., Wabinga H. and Whelan S.L. (2003) Cancer in Africa: Epidemiology and Prevention. 4.3 Cervix Cancer. . In *IARC Scientific Publications* pp. 268–276. Lyon.

Petrosky E., Bocchini Jr J.A., Hariri S., Chesson H., Curtis C.R., Saraiya M., Unger E.R. and Markowitz L.E. (2015) Use of 9-valent human papillomavirus (HPV) vaccine: updated HPV vaccination recommendations of the Advisory Committee on Immunization Practices. *MMWR* 64, 300-304.

Petry K.U., Luyten A., Justus A., Iftner A., Strehlke S., Schulze-Rath R. and Iftner T. (2012) Prevalence of low-risk HPV types and genital warts in women born 1988/89 or 1983/84-results of WOLVES, a population-based epidemiological study in Wolfsburg, Germany. *BMC infectious diseases* 12, 367.

Peyton C.L., Gravitt P.E., Hunt W.C., Hundley R.S., Zhao M., Apple R.J. and Wheeler C.M. (2001) Determinants of genital human papillomavirus detection in a US population. *Journal of Infectious Diseases* 183, 1554-1564.

Pirog E.C., Lloveras B., Molijn A., Tous S., Guimerà N., Alejo M., Clavero O., Klaustermeier J., Jenkins D. and Quint W.G. (2014) HPV prevalence and genotypes in different histological subtypes of cervical adenocarcinoma, a worldwide analysis of 760 cases. *Modern Pathology* 27, 1559.

Plummer M., Schiffman M., Castle P.E., Maucort-Boulch D. and Wheeler C.M. (2007) A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. *Journal of Infectious Diseases* 195, 1582-1589.

Quint W., Jenkins D., Molijn A., Struijk L., van de Sandt M., Doorbar J., Mols J., Van Hoof C., Hardt K., Struyf F. and Colau B. (2012) One virus, one lesion--individual components of CIN lesions contain a specific HPV type. *The Journal of pathology* 227, 62-71.

Richter K., Becker P., Horton A. and Dreyer G. (2013) Age-specific prevalence of cervical human papillomavirus infection and cytological abnormalities in women in Gauteng Province, South Africa. *SAMJ: South African Medical Journal* 103, 313-317.

Rousseau M.C., Pereira J.S., Prado J.C., Villa L.L., Rohan T.E. and Franco E.L. (2001) Cervical coinfection with human papillomavirus (HPV) types as a predictor of acquisition and persistence of HPV infection. *Journal of Infectious Diseases* 184, 1508-1517.

Rubin M.A., Kleter B., Zhou M., Ayala G., Cubilla A.L., Quint W.G. and Pirog E.C. (2001) Detection and typing of human papillomavirus DNA in penile carcinoma: evidence for multiple independent pathways of penile carcinogenesis. *The American journal of pathology* 159, 1211-1218.

Sahasrabuddhe V., Mwanahamuntu M.H., Vermund S., Huh W., Lyon M., Stringer J. and Parham G. (2007) Prevalence and distribution of HPV genotypes among HIV-infected women in Zambia. *British Journal of Cancer* 96, 1480-1483.

Sarkar K., Pal R., Bal B., Saha B., Bhattacharya S., Sengupta S., Mazumdar P.P. and Chakraborti S. (2011) Oncogenic HPV among HIV infected female population in West Bengal, India. *BMC infectious diseases* 11, 72.

Saslow D., Solomon D., Lawson H.W., Killackey M., Kulasingam S.L., Cain J., Garcia F.A., Moriarty A.T., Waxman A.G. and Wilbur D.C. (2012) American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer. *CA: a cancer journal for clinicians* 62, 147-172.

SCHEURER M.E., Tortolero-Luna G. and Adler-Storthz K. (2005) Human papillomavirus infection: biology, epidemiology, and prevention. *International Journal of Gynecologic Cancer* 15, 727-746.

Schiffman M., Castle P.E., Maucort-Boulch D., Wheeler C.M. and Plummer M. (2007) A 2-year prospective study of human papillomavirus persistence among women with a cytological diagnosis of atypical squamous cells of undetermined significance or low-grade squamous intraepithelial lesion. *Journal of Infectious Diseases* 195, 1582-1589.

Schiffman M.H., Bauer H.M., Hoover R.N., Glass A.G., Cadell D.M., Rush B.B., Scott D.R., Sherman M.E., Kurman R.J., Wacholder S., Stanton C.K. and Manos M.M. (1993) Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. *Journal of the National Cancer Institute* 85, 958-964.

Schlecht N.F., Kulaga S., Robitaille J., Ferreira S., Santos M., Miyamura R.A., Duarte-Franco E., Rohan T.E., Ferenczy A., Villa L.L. and Franco E.L. (2001) Persistent human papillomavirus infection as a predictor of cervical intraepithelial neoplasia. *JAMA* 286, 3106-3114.

Schulze M.H., Völker F.M., Lugert R., Cooper P., Hasenclever K., Groß U., Pfister H. and Silling S. (2016) High prevalence of human papillomaviruses in Ghanaian pregnant women. *Medical microbiology and immunology* 205, 595-602.

Shah U.J., Nasiruddin M., Dar S.A., Khan M.K.A., Akhter M.R., Singh N., Rabaan A.A. and Haque S. (2020) Emerging biomarkers and clinical significance of HPV genotyping in prevention and management of cervical cancer. *Microbial Pathogenesis* 143, 104131.

Sieber P., Platzer M. and Schuster S. (2018) The definition of open reading frame revisited. *Trends in Genetics* 34, 167-170.

Sinayobye J.d.A., Sklar M., Hoover D.R., Shi Q., Dusingize J.C., Cohen M., Mutimura E., Asiimwe-Kateera B., Castle P.E. and Strickler H. (2014) Prevalence and risk factors for High-Risk Human Papillomavirus (hrHPV) infection among HIV-infected and Uninfected Rwandan women: implications for hrHPV-based screening in Rwanda. *Infectious agents and cancer* 9, 40.

Sloan F.A. and Gelband H. (2007) The cancer burden in low-and middle-income countries and how it is measured. In *Cancer Control Opportunities in Low-and Middle-Income Countries*: National Academies Press (US).

Soccio J., Brown M., Comino E. and Friesen E. (2015) Pap smear screening, pap smear abnormalities and psychosocial risk factors among women in a residential alcohol and drug rehabilitation facility. *Journal of advanced nursing* 71, 2858-2866.

Solomon D., Davey D., Kurman R., Moriarty A., O'Connor D., Prey M., Raab S., Sherman M., Wilbur D. and Wright Jr T. (2002) The 2001 Bethesda System: terminology for reporting results of cervical cytology. *JAMA* 287, 2114-2119.

Sotlar K., Diemer D., Dethleffs A., Hack Y., Stubner A., Vollmer N., Menton S., Menton M., Dietz K., Wallwiener D., Kandolf R. and Bultmann B. (2004) Detection and typing of human papillomavirus by e6 nested multiplex PCR. *Journal of Clinical Microbiology*
42, 3176-3184.

Strickler H.D., Burk R.D., Fazzari M., Anastos K., Minkoff H., Massad L.S., Hall C., Bacon M., Levine A.M. and Watts D.H. (2005) Natural history and possible reactivation of human papillomavirus in human immunodeficiency virus–positive women. *Journal of the National Cancer Institute* 97, 577-586.

Sun X.W., Kuhn L., Ellerbrock T.V., Chiasson M.A., Bush T.J. and Wright T.C., Jr. (1997) Human papillomavirus infection in women infected with the human immunodeficiency virus. *New England Journal of Medicine* 337, 1343-1349.

Tao L., Han L., Li X., Gao Q., Pan L., Wu L., Luo Y., Wang W., Zheng Z. and Guo X. (2014) Prevalence and risk factors for cervical neoplasia: a cervical cancer screening program in Beijing. *BMC Public Health* 14, 1185.

Thomas J., Ojemakinde K., Ajayi I., Omigbodun A., Fawole O. and Oladepo O. (2012) Population-based prevalence of abnormal cervical cytology findings and local risk factors in Ibadan, Nigeria: implications for cervical cancer control programs and human papilloma virus immunization. *Acta Cytol* 56, 251-258.

Thomas K.K., Hughes J.P., Kuypers J.M., Kiviat N.B., Lee S.K., Adam D.E. and Koutsky L.A. (2000) Concurrent and sequential acquisition of different genital human papillomavirus types. *Journal of Infectious Diseases* 182, 1097-1102.

Toki T., Kurman R.J., Park J.S., Kessis T., Daniel R.W. and Shah K.V. (1991) Probable nonpapillomavirus etiology of squamous cell carcinoma of the vulva in older women: a clinicopathologic study using in situ hybridization and polymerase chain reaction. *International journal of gynecological pathology: official journal of the International Society of Gynecological Pathologists* 10, 107-125.

Tornesello M.L., Giorgi Rossi P., Buonaguro L. and Buonaguro F.M. (2014) Human papillomavirus infection and cervical neoplasia among migrant women living in Italy. *Frontiers in oncology* 4, 31.

Torre L.A., Siegel R.L., Ward E.M. and Jemal A. (2016) Global cancer incidence and mortality rates and trends—an update. *Cancer Epidemiology and Prevention Biomarkers* 25, 16-27.

Trottier H. and Franco E.L. (2006) The epidemiology of genital human papillomavirus infection. *Vaccine* 24, S4-S15.

Trottier H., Mahmud S., Costa M.C., Sobrinho J.P., Duarte-Franco E., Rohan T.E., Ferenczy A., Villa L.L. and Franco E.L. (2006a) Human papillomavirus infections with multiple types and risk of cervical neoplasia. *Cancer Epidemiology and Prevention Biomarkers* 15, 1274-1280.

Trottier H., Mahmud S., Costa M.C., Sobrinho J.P., Duarte-Franco E., Rohan T.E., Ferenczy A., Villa L.L. and Franco E.L. (2006b) Human papillomavirus infections with multiple types and risk of cervical neoplasia. *Cancer Epidemiology Biomarkers & Prevention* 15, 1274-1280.

Turek L.P. (1994) The structure, function, and regulation of papillomaviral genes in infection and cervical cancer. *Adv Virus Res* 44, 305-356.

Unaids (2010) *AIDS Epidemic Update November 2009*: World Health Organization.

Vaccarella S., Herrero R., Dai M., Snijders P.J., Meijer C.J., Thomas J.O., Hoang Anh P.T., Ferreccio C., Matos E., Posso H., de Sanjose S., Shin H.R., Sukvirach S., Lazcano-Ponce E., Ronco G., Rajkumar R., Qiao Y.L., Munoz N. and Franceschi S. (2006) Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. *Cancer Epidemiology Biomarkers & Prevention* 15, 2148-2153.

van den Brule A.J., Pol R., Fransen-Daalmeijer N., Schouls L.M., Meijer C.J. and Snijders P.J. (2002) GP5+/6+ PCR followed by reverse line blot analysis enables rapid and high-throughput identification of human papillomavirus genotypes. *Journal of Clinical Microbiology*
40, 779-787.

Vera-Iglesias E., García-Arpa M., Sánchez-Caminero P., Romero-Aguilera G. and de la Calle P.C. (2007) Focal epithelial hyperplasia. *Actas Dermo-Sifiliográficas (English Edition)* 98, 621-623.

Villa L.L., Costa R.L., Petta C.A., Andrade R.P., Ault K.A., Giuliano A.R., Wheeler C.M., Koutsky L.A., Malm C., Lehtinen M., Skjeldestad F.E., Olsson S.E., Steinwall M., Brown D.R., Kurman R.J., Ronnett B.M., Stoler M.H., Ferenczy A., Harper D.M., Tamms G.M., Yu J., Lupinacci L., Railkar R., Taddeo F.J., Jansen K.U., Esser M.T., Sings H.L., Saah A.J. and Barr E. (2005) Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *The Lancet Oncol* 6, 271-278.

Wabinga H., Parkin D., Wabwire-Mangen F. and Nambooze S. (2000) Trends in cancer incidence in Kyadondo County, Uganda, 1960–1997. *British Journal of Cancer* 82, 1585.

Walboomers J., Jacobs M., Manos M., Bosch F., Kummer J., Shah K., Snijders P., Peto J., Meijer C. and Munoz N. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *The Journal of pathology* 189, 12 - 19.

Wallin K.L., Wiklund F., Angstrom T., Bergman F., Stendahl U., Wadell G., Hallmans G. and Dillner J. (1999) Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *New England Journal of Medicine* 341, 1633-1638.

Wandeler G., Johnson L.F. and Egger M. (2016) Trends in life expectancy of HIV-positive adults on ART across the globe: comparisons with general population. *Current Opinion in HIV and AIDS* 11, 492.

Wang S.S., Gonzalez P., Yu K., Porras C., Li Q., Safaeian M., Rodriguez A.C., Sherman M.E., Bratti C., Schiffman M., Wacholder S., Burk R.D., Herrero R., Chanock S.J. and Hildesheim A. (2010) Common genetic variants and risk for HPV persistence and progression to cervical cancer. *PLoS One* 5, e8667.

Wanichwatanadecha P., Sirisrimangkorn S., Kaewprag J. and Ponglikitmongkol M. (2012) Transactivation activity of human papillomavirus type 16 E6* I on aldo-keto reductase genes enhances chemoresistance in cervical cancer cells. *Journal of General Virology* 93, 1081-1092.

Wanyoike-Gichuhi J., Kayumba P. and Khisa W. (2014) Prevalence of cervical cytology abnormalities among HIV infected women at Rwanda Military Hospital. *East African medical journal* 91, 333-340.

WHO (1999) The current status of development of prophylactic vaccines against human papillomavirus infection. Report of a technical meeting. : World Health Organization;.

WHO/ICO (2010a) Human Papillomavirus and Related Cancers. In *WHO Information Center on HPV and Cervical Cancer (HPV Information Centre)* WHO/ICO.

WHO/ICO (2010b) Human Papillomavirus and Related Cancers In the World. In *WHO Information Center on HPV and Cervical Cancer (HPV Information Centre)* WHO/ICO.

Winer R.L. and Koutsky L.A. (2004) The epidemiology of human papillomavirus infections. In *Cervical cancer: from etiology to prevention*, pp. 143–187 [T. Rohan and K. Shah, editors]. Dordrecht, The Netherlands: Kluwer Academic Publishers.

Winer R.L., Lee S.K., Hughes J.P., Adam D.E., Kiviat N.B. and Koutsky L.A. (2003) Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. *Am J Epidemiol* 157, 218-226.

Wiredu E.K. and Armah H.B. (2006) Cancer mortality patterns in Ghana: a 10-year review of autopsies and hospital mortality. *BMC Public Health* 6, 159.

Womack C., Turner A.G. and Fisher C. (2000) Paratesticular liposarcoma with smooth muscle differentiation mimicking angiomyolipoma. *Histopathology* 36, 221-223.

Woodman C.B., Collins S., Winter H., Bailey A., Ellis J., Prior P., Yates M., Rollason T.P. and Young L.S. (2001) Natural history of cervical human papillomavirus infection in young women: a longitudinal cohort study. *The Lancet* 357, 1831-1836.

Woodman C.B., Collins S.I. and Young L.S. (2007) The natural history of cervical HPV infection: unresolved issues. *Nat Rev Cancer* 7, 11-22.

Wright T.C., Stoler M.H., Behrens C.M., Sharma A., Sharma K. and Apple R. (2014) Interlaboratory variation in the performance of liquid- based cytology: Insights from the ATHENA trial. *International journal of cancer* 134, 1835-1843.

Xi L.F., Toure P., Critchlow C.W., Hawes S.E., Dembele B., Sow P.S. and Kiviat N.B. (2003) Prevalence of specific types of human papillomavirus and cervical squamous intraepithelial lesions in consecutive, previously unscreened, West-African women over 35 years of age. *International journal of cancer* 103, 803-809.

Yakasai I., Abdullahi H., Muhammed A. and Galadanci H. (2012) Prevalence of Cervical Dysplasia among Women in Kano Municipal Kano State, Nigeria. *Journal of Medicine in the Tropics* 14, 64-68.

Yar D.D., Salifu S.P., Darko S.N., Annan A.A., Gyimah A.A., Buabeng K.O. and Owusu- Dabo E. (2015) Genotypic characterisation of human papillomavirus infections among persons living with HIV infection; a case–control study in Kumasi, Ghana. *Tropical Medicine & International Health*.

Yar D.D., Salifu S.P., Darko S.N., Annan A.A., Gyimah A.A., Buabeng K.O. and Owusu- Dabo E. (2016) Genotypic characterisation of human papillomavirus infections among persons living with HIV infection; a case–control study in Kumasi, Ghana. *Tropical Medicine & International Health* 21, 275-282.

Yu L., Majerciak V. and Zheng Z.-M. (2022) HPV16 and HPV18 Genome Structure, Expression, and Post-Transcriptional Regulation. *International Journal of Molecular Sciences* 23, 4943.

Zeier M., Nachega J., Van Der Merwe F., Eshun-Wilson I., Van Schalkwyk M., La Grange M., Mason D., Louw M. and Botha M. (2012) Impact of timing of antiretroviral therapy initiation on survival of cervical squamous intraepithelial lesions: a cohort analysis from South Africa. *Int J STD AIDS* 23, 890-896.

Zhao X.-L., Hu S.-Y., Zhang Q., Dong L., Feng R.-M., Han R. and Zhao F.-H. (2017) High-risk human papillomavirus genotype distribution and attribution to cervical cancer and precancerous lesions in a rural Chinese population. *Journal of gynecologic oncology* 28.

Zheng Z.-M. (2014) Human papillomaviruses. In *Cancers in People with HIV and AIDS*, pp. 87-112: Springer.

Zheng Z.-M. and Baker C.C. (2006) Papillomavirus genome structure, expression, and post-transcriptional regulation. *Frontiers in bioscience: a journal and virtual library* 11, 2286.

Zhu Y., Wang Y., Hirschhorn J., Welsh K.J., Zhao Z., Davis M.R. and Feldman S. (2017) Human papillomavirus and its testing assays, cervical cancer screening, and vaccination. *Advances in clinical chemistry* 81, 135-192.

Zielinski G.D., Bais A.G., Helmerhorst T.J., Verheijen R.H., de Schipper F.A., Snijders P.J., Voorhorst F.J., van Kemenade F.J., Rozendaal L. and Meijer C.J. (2004) HPV testing and monitoring of women after treatment of CIN 3: review of the literature and meta-analysis. *Obstetrical & gynecological survey* 59, 543-553.

zur Hausen H. (1996) Papillomavirus infections--a major cause of human cancers. *Biochim Biophys Acta* 1288, F55-78.

zur Hausen H. (2002) Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2, 342-350.

APPENDIX 1

PATIENT QUESTIONNAIRE

Project Title: Genotypes of Human Papillomavirus among HIV Positive Women Attending Clinic at the Korle-bu Teaching Hospital, Accra, Ghana

Subject No…… Date……..

Personal Profile

1. Age……
2. Educational Level: None [ ] Primary [ ] Secondary[ ] Tertiary [ ]
3. Occupation…….
4. Residence……..
5. Region……
6. Ethnicity…….
7. Marital status: Single[ ] Married[ ] Separated[ ] Divorced[ ] Widowed
8. How do you pay your medical bills? Self [ ] Relatives[ ] Employer[ ] NHIS

Risk Factors for HPV infection

Sexual Partners

9. Are you the only wife of your husband? Yes[ ] No[ ]
10. If no how many wives does your husband have? .....
11. How many permanent sexual partners do you have? .....
12. How many sexual partners have you had in the past 6months? ……
13. How many lifetime sexual partners have you had? ……
14. At what age did you first have sex? .....

15. How often do you have sex? .....

History on Pregnancy

16. Have you ever been pregnant? Yes [ ] No [ ]

If yes, what was your age at first pregnancy? .....

How many times have you been pregnant? .....

How many of your pregnancies resulted in miscarriage or abortion? .....

How many of your pregnancies resulted in still birth? .....

Smoking status

17. Do you smoke tobacco? Yes [ ] No[ ]

If yes, how long have you been smoking? .....

How many cigarettes on average do you smoke per week? .....

18. Have you smoked before? Yes [ ] No[ ]

19. If yes how long did you smoke? .....

20. Do you use any other tobacco-related product? (snuff) Yes [ ] No[ ]

If yes, how long have you been using it? .....

How many tobacco-related products on average do you smoke per week? .....

Condom Use

21. Do you use condoms during sex? Always[ ] Sometimes[ ]Never[ ]

Other Sexually Transmitted Infection

when were you diagnosed of HIV? .....

22. Are you using anti-retroviral therapy? Yes [ ] No [ ]

23. If yes, how long have you been using it?.....

24. Do you have any sexually transmitted infection? Yes[ ] No[ ]

If yes, state what type it is.....

When was it diagnosed? .....

25. Have you been infected with any other sexually transmitted infection before?

Yes [ ] No [ ]

26. If yes, state what type it was.....

When was it diagnosed? .....

Subject number: ……..

CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Genotypes of Human Papillomavirus among HIV Positive Women Attending clinic at the Korle bu Teaching Hospital, Accra, Ghana

Before agreeing to participate in this research study, it is important that you read the following explanation of this study. This statement describes the purpose, procedures, benefits, risks, discomforts, and precautions of the program. Also described are the alternative procedures available to you, as well as your right to withdraw from the study at any time.

Explanation of Procedures

You are being invited to participate in a research project to determine the prevalence and genotypes (the type of Human Papillomavirus found) of HPV infection among women living with HIV in Accra. The approach of the research is through the use of a questionnaire and cervical specimen collection. You will complete the questionnaire that will require answers on personal profile and risk factors for HPV infection (i.e. number of sexual partners, smoking status, contraceptive use etc.). Afterwards, you will be examined by medical officer and cervical specimen will be collected by inserting a speculum into your vagina, which spreads the vagina open and allows access to the cervix. A sample of cells is then collected from the outer opening or os of the cervix by scraping with an Aylesbury disposable spatula. An endocervical brush is rotated in the central opening of the cervix. This procedure will be carried out in a private room with your comfort and safety in mind. Your cell phone numbers will be collected so that we can contact you if need be for treatment or management purposes.

Risks and Discomforts

By participating in this research, you are likely to experience some form of discomfort. This includes the discomfort of questioning, physical examination, and the pain of cervical specimen collection. The team will try and decrease your chances of these risks from occurring, but if an untoward event happens, they will provide you with free medical care.

Benefits

Women who are found to have genital warts, Cervical Intraepithelial Neoplasia (CIN) and cervical cancer shall be called back for treatment and management. However, this research is expected to provide data on HPV genotypes and distribution for policy makers when vaccination becomes a mainstream policy in Ghana.

Confidentiality

All information gathered from the study will remain confidential. Your identity as a participant will not be disclosed to any unauthorized persons; only the researchers, Ghana Health Service and School of Allied Health Science will have access to the research materials, which will be kept under lock. Any references to your identity that would compromise your anonymity will be removed or disguised prior to the preparation of the research reports and publications.

Withdrawal from Project

Participation in this study is voluntary; refusal to participate will involve no penalty. You are free to withdraw consent and discontinue participation in this project at any time without prejudice from the research team.

Costs and/or Payments to Subject for Participation in Research

There will be no costs for participating in the research. Also, you will not be paid to participate in this research project.

Any questions concerning the research project and/or in the case of injury due to the project, participants can call Professor E. K Wiredu, School of Allied health Sciences (0244664184) or Dodzi Kofi Amelor of the School of Public Heath (0266876544).

Questions regarding any rights issues as a person in this research project should be directed to the chairpersons of the Ethical Review Committees of the Korle-bu Teaching Hospital and School of Allied Health Science respectively.

Consent to participate in Research

I, ……

□ Confirm that I have read the written information (or have had the information read to me) for the study :**Genotypes of Human Papillomavirus among HIV Positive**

Women Attending clinic at the Korle bu Teaching Hospital, Accra, Ghana and that the study procedures have been explained to me by study staff during the consent process for this study.

 Confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided.

 Understand that I grant access to data to authorised persons described in the information sheet.

 Have been given time and opportunity to consider taking part in this study.

Tick as appropriate (this decision will not affect your ability to enter the study):

Thumb of subject unable to sign

I consent to participate in the above research study.

Signature of Subject: .....

Date.....

Signature of Interviewer: .....

Date.....

Name of

Impartial Witness: ……

Signature of

Impartial Witness: …….

Date.....

APPENDIX 2

Appendix 1:

Protocol: DNA Purification from Cervical Swabs using Qiagen DNA Mini kit (Spin Method)

1. Centrifuge sample at 5000 rpm for 5 min.
2. Add 20 µl QIAGEN Proteinase K and 300 µl Buffer AL to the sample. Mix immediately by vortexing for 15 s.
   NB: Do not add Qiagen Proteinase K directly to Buffer AL.
3. Centrifuge at 5000rpm for 5 min and separate filtrate into 1.5ml microcentrifuge tubes.
4. Incubate at 56°C for 10 min.
5. Add 400 µl ethanol (96–100%) to the sample and mix again by vortexing. Briefly centrifuge to remove drops from inside the lid.
6. Carefully apply 700 µl of the mixture from step 4 to the QIAamp Mini spin column (in a 2 ml collection tube) without wetting the rim. Close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.*
7. Repeat step 6 by applying up to 700 µl of the remaining mixture from step 5 to the QIAamp Mini spin column.
8. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp Mini spin column in a clean 2 ml collection tube, and discard the collection tube containing the filtrate.*
9. Carefully open the QIAamp Mini spin column and add 500 µl Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.

Recommended: Place the QIAamp Mini spin column in a new 2 ml collection tube and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 min.

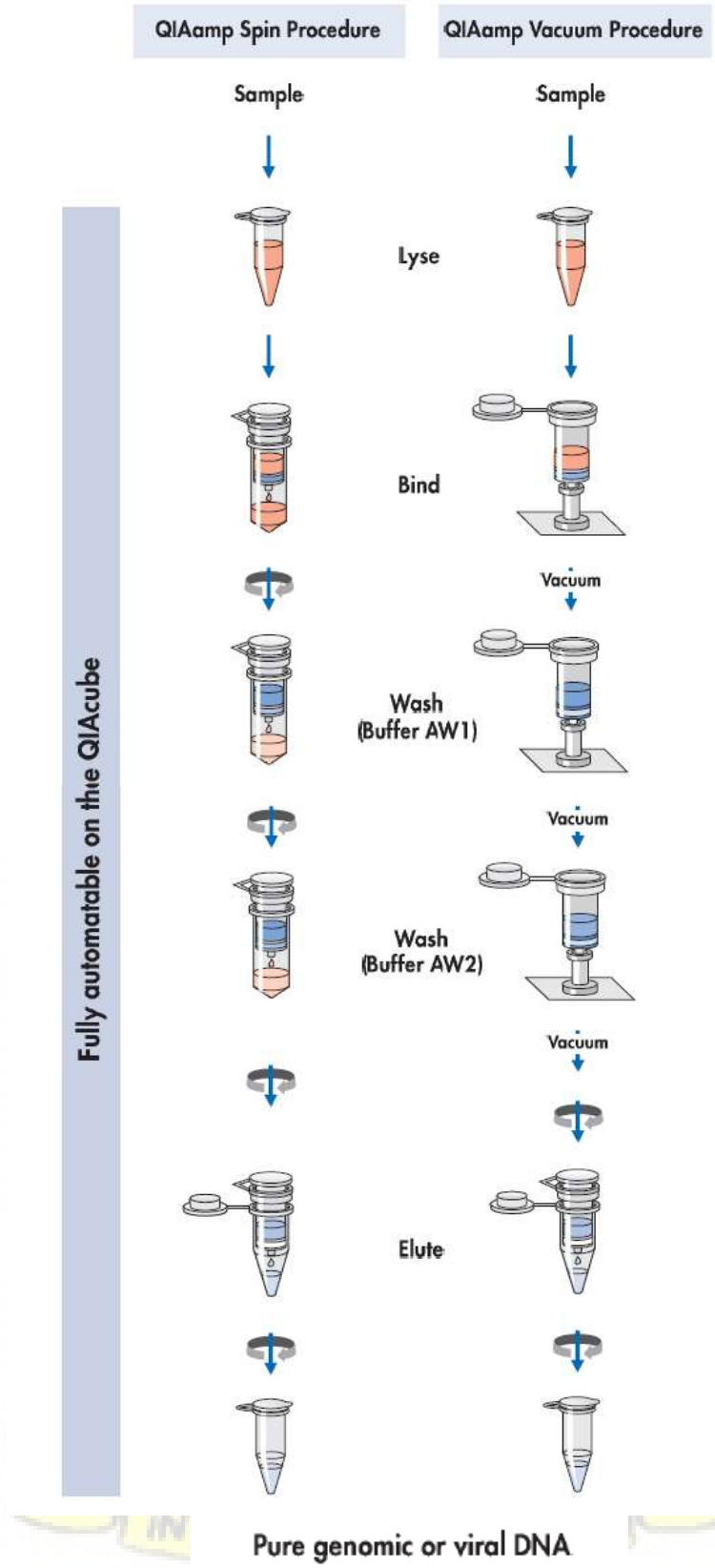
10. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate.

11. Carefully open the QIAamp Mini spin column and add 50 µl Buffer AE. Incubate at room temperature for 5 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.

12. Aliquot 25 µl portions of eluate into 1.5ml microcentrifuge tubes on ice. Label with sample code and date. Store at -20^{o}C.

Precautions:

**Flow-through contains Buffer AL or Buffer AW1 and is therefore not compatible with bleach.*



Adapted from QIAamp DNA Mini and Blood Mini Handbook 04/2010

APPENDIX 3

Appendix 2
DNA sequence detail of oligonucleotide primers used for HPV genotyping

Adapted from Sotlar *et. al.,* (2004).

Primer cocktail	HPV genotype	Amplicon (bp)	Sequence (5'-3')	Position (bp)
I	16	457	CAC AGT TAT GCA CAG AGC TGC	141–161
			CAT ATA TTC ATG CAA TGT AGG TGT A	597–573
	18	322	CAC TTC ACT GCA AGA CAT AGA	170–190
			GTT GTG AAA TCG TCG TTT TTC A	491–470
	31	263	GAA ATT GCA TGA ACT AAG CTC G	137–158
			CAC ATA TAC CTT TGT TTG TCA A	399–378
	59	215	CAA AGG GGA ACT GCA AGA AAG	159–179
			TAT AAC AGC GTA TCA GCA GC	373–354
	45	151	GTG GAA AAG TGC ATT ACA GG	82–101
			ACC TCT GTG CGT TCC AAT GT	232–213
II	33	398	ACT ATA CAC AAC ATT GAA CTA	172–192
			GTT TTT ACA CGT CAC AGT GCA	569–549
	6/11	334	TGC AAG AAT GCA CTG ACC AC	201–220
			TGC ATG TTG TCC AGC AGT GT	534–515
	58	274	GTA AAG TGT GCT TAC GAT TGC	297–317
			GTT GTT ACA GGT TAC ACT TGT	570–550
	52	229	TAA GGC TGC AGT GTG TGC AG	178–197
			CTA ATA GTT ATT TCA CTT AAT GGT	406–383
	56	181	GTG TGC AGA GTA TGT TTA TTG	294–314
			TTT CTG TCA CAA TGC AAT TGC	475–455
III	35	358	CAA CGA GGT AGA AGA AAG CAT C	157–178
			CCG ACC TGT CCA CCG TCC ACC G	514–493
	42	277	CCC AAA GTA GTG GTC CCA GTT A	85–106
			GAT CTT TCG TAG TGT CGC AGT G	361–340
	43	219	GCA TAA TGT CTG CAC GTA GCT G	102–123
			CAT GAA ACT GTA GAC AGG CCA AG	320–298
	44	163	TAA ACA GTT ATA TGT AGT GTA CCG	248–271
			TAT CAG CAC GTC CAG AAT TGA C	410–389
IV	68	333	GCA GAA GGC AAC TAC AAC GG	4049–4068
			GTT TAC TGG TCC AGC AGT GG	4381–4362
	39	280	GAC GAC CAC TAC AGC AAA CC	213–232
			TTA TGA AAT CTT CGT TTG CT	492–473
	51	223	GAG TAT AGA CGT TAT AGC AGG	319–339
			TTT CGT TAC GTT GTC GTG TAC G	541–520
	66	172	TTC AGT GTA TGG GGC AAC AT	353–372
			AAA CAT GAC CCG GTC CAT GC	520–501

APPENDIX 4

Appendix 3

APPENDIX 5

Table 6.1-1: Combinations of human papillomavirus (HPV) types in women with multiple infections

Double		Triple		Four		Five or more	
HPV cluster	**frequency**	**HPV cluster**	**frequency**	**HPV cluster**	**frequency**	**HPV cluster**	**frequency**
16,18	2	**16,42,44**		**16,18,6/11,35**		**16,18,35,42,58**	2
16,33		**18,33,42**		**16,6/11,43,51**		**18,35,43,58,68**	
16,35		**18,35,39**		**18,33,35,52**		**45,6/11,56,43,35**	
18,33		**18,35,42**	2	**18,35,42,52**		**18,6/11,35,42,45,52**	
18,35	12	**18,35,58**	2	**18,35,42,68**		**16,18,35,42,45,58,68**	
18,45		**18,35,68**		**18,42,52,68**			
18,52	2	**18,42,39**		**18,52,58,35**			
18,58	2	**18,42,45**		**18,6/11,33,35**			
18,6/11	4	**18,42,58**		**18,6/11,35,58**	2		
18,68		**18,43,52**		**35,45,52,56**			
31,35		**18,45,58**		**42,44,51,66**			
31,43		**18,52,68**		**45,58,43,51**			
35,39		**18,58,68**	2	**56,52,35,68**			
35,45		**18,6/11,35**		**6/11,18,52,68**			
35,51		**31,43,52**	3				
35,52	3	**35,42,39**					
35,58	2	**35,45,52**					
35,68	2	**35,51,58**					
39,45	2	**35,52,68**					
39,58		**43,45,51**					
42,51		**44,66,68**	2				

Table x continued

Double		Triple		Four		Five or more	
HPV cluster	**frequency**	**HPV cluster**	**frequency**	**HPV cluster**	**frequency**	**HPV cluster**	**frequency**
42,58	2	**45,58,44**					
42,59	2	**52,56,43**					
42,68		**58,35,68**					
43,39		**6/11 ,16,35**					
43,51		**6/11,39,43**	2				
43,58	3	**6/11,52,58**					
43,68							
45,52	2						
45,58							
45,68	2						
52,42	2						
52,44							
52,68							
56, 58							
59,16							
59,51							
59,52							
6/11 , 45							
6/11,31							
6/11,43	2						
6/11,68	3						

Unless otherwise stated in the frequency column these clusters are all single occurrences.