DISTRIBUTION OF HUMAN PAPILLOMAVIRUS (HPV) GENOTYPES AND ASSOCIATED CERVICAL DISEASE IN AN UNSCREENED POPULATION OF WOMEN IN KUMASI, GHANA

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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

BACKGROUND

Cervical cancer is the commonest gynaecologic cancer and second leading cause of all cancers in Ghana. High-risk human papillomavirus infection is a necessary cause of cervical cancer and pre-cursor lesions. However, little is known about the risk of human papillomavirus infection and potential benefit from available vaccines in Ghana.

STUDY SETTING

We designed a cross-sectional descriptive study to establish the distribution of genital HPV genotypes among an unscreened population of women recruited from three cervicare centers in Kumasi, Ghana. Cervical swabs were available for 593 eligible women from May 2012 to November 2014.

METHODS

Cervical swabs were carried in DNA Guard solution according to the manufacturer's instruction until DNA extraction using the QIAamp DNA Mini kit. Purified DNA was stored at -70ºC in duplicate. A nested multiplex PCR (NMPCR) assay that combines degenerate E6/E7 consensus primers and type-specific primers was utilized for the detection and typing of human papillomavirus (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 by two cytotechnologists and confirmed by a pathologist. Participants were required to answer some questions relating to their sexual and reproductive habits as well.

RESULTS

HPV E6/E7 oncogenic DNA was detected in 37.2% of all cervical swabs tested. The prevalence of HPV among women with normal and abnormal cytology was 35.7% and 62.9% respectively. High-risk HPV was detected in all suspected cancer cases (SCC), highgrade squamous intraepithelial lesions (HSILs), low-grade squamous intraepithelial lesions (LSILs), and atypical squamous cells of undetermined significance (ASCUS). Overall, the commonest HR types detected were HPV 52, 56, 35, 18, 58, 68, 51, 39, 45 and 16 in decreasing order. The commonest HPV genotypes detected among women with ASCUS were HPV-18, 52 and 68 (25% each of ASCUS); among women with LSIL were HPV-52 (66.5% of LSILS), HPV-18 (22.2%) and HPV 45 (22.2%). Among high-grade

dysplasia (HSIL), the commonest types were HPV-16, 52 and 42 (50% each). HPV-16 (66.6% of SCC) was more commonly detected in cases with suspected squamous cell carcinoma than any other genotype. HPV positivity was associated with sexual history variables: having a history of multiple sexual partners and polygamous sexual arrangements.

CONCLUSION

The present study has demonstrated HPV E6 and E7 oncogenes in a large fraction of Ghanaian women attending gynaecological screening in Kumasi. There is a preponderance of multiple HPV infections in the population studied. The study has established that HPV 16 and 18 are not the commonest HPV types in the general population of women as previously perceived. However, the relative fractions of HPV 16 and 18 increases with severity of cervical lesions indicating the more aggressive nature of their E6 and E7 gene products. The study has furthermore shown that in future, cases of HPV 52 infection might become more significant in this population as well.

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ABBREVIATIONS

χ^2	Chi-square statistic
β	Regression coefficient
ACIP	Advisory Committee on Immunization Practices
AIS	adenocarcinoma in-situ
ASC-H:	Atypical squamous cells, cannot rule out HSIL
ASC-US:	Atypical squamous cells of undetermined significance
BPV	Bovine papillomavirus

CDKN2A:	Cyclin-dependent kinase inhibitor 2A
CIN:	Cervical intraepithelial neoplasia
CIS:	Carcinoma in-situ
CMI	cell-mediated immunity
CT:	Chlamydia Trachomatis
df	Degrees of freedom
DNA:	Deoxyribonucleic acid
GAVI	Global Alliance for Vaccines Initiative
GLOBOCAN	Global Cancer Network
GSS	Ghana Statistical Service
HGCIN:	high-grade cervical intraepithelial neoplasia
HIV:	Human immunodeficiency virus
HLA	Human Leukocyte Antigen
HLA:	human leukocyte antigen
HSIL:	high grade squamous intraepithelial lesion
HSV:	Herpes simplex virus
IARC:	International Agency for Research on Cancer
ICC	Invasive Cervical Cancer
ICTV	International Committee on the Taxonomy of Viruses
IgA:	Immunoglobulin alpha
IgG:	Immunoglobulin gamma
KSRH:	Kumasi South Regional Hospital
LCR	long control region
LSIL:	low-grade squamous intraepithelial lesion
MCM2:	Minichromosome maintenance protein
nmPCR:	Nested multiplex polymerase chain reaction
OPD	Out-patient Department
ORF:	open reading frame
pA	polyadenylation
PCR:	polymerase chain reaction
SCC:	Squamous cell carcinoma

SIL:	Squamous intraepithelial lesions
Top2A:	Topoisomerase IIA
URR :	Upstream regulatory region
VIA:	Visual inspection with acid
VLP	Virus-like particle
WHO:	World Health Organization
YLL	Years of life lost
PPS	Probability-proportional-to-size sampling
WIFA	Women of fertility age
KMHD	Kumasi Metropolitan Health Directorate
CHRPE	Committee on Human Research, Publication and Ethics
GHS	Ghana Health Service
KNUST	Kwame Nkrumah University of Science and Technology,
SMS	School of Medical Sciences
SAHS	School of Allied Health Sciences
NTZC	No transformation zone components
NDS	No dyskaryosis seen
LR-HPV	Low-risk human papillomavirus
HR-HPV	High-risk human papillomavirus

Chapter 1 INTRODUCTION

1.1 BACKGROUND

Cancer is the leading cause of death in economically developed countries and the second leading cause of death in developing countries (Arbyn *et al.*, 2011). Based on the GLOBOCAN 2012 estimates, there were 14.1 million new cancer cases, 8.2 million cancer deaths and 32.6 million people living with cancer (within 5 years of diagnosis) in 2012 worldwide. Fifty-seven percent (8 million) of new cancer cases, 65% (5.3 million) of the cancer deaths and 48% (15.6 million) of the 5-year prevalent cancer cases occurred in the less developed regions (Ferlay *et al.*, 2012). According to global statistics, invasive cervical cancer (ICC) ranks joint third with lung cancer as the most common type of cancer after breast cancer (Arbyn *et al.*, 2011). With an estimated 530,000 new cases and nearly 275,000 deaths occurring each year, cervical cancer is the fourth most common cause of death due to cancer in women (Arbyn *et al.*, 2011). The burden of ICC is also disproportionately high in developing countries. Almost nine out of ten (87%) cervical cancer deaths occur in these countries. Mortality varies 18-fold between the different regions of the world, with rates ranging from less than 2 per 100,000 in Western Asia, Western Europe and Australia/New Zealand to more than 20 per 100,000 in Melanesia (20.6), Middle (22.2) and Eastern (27.6) Africa (Ferlay *et al.*, 2012).

In 2006, Wiredu and Armah (2006) reported a 10-year review of autopsies and hospital mortality in which cervical cancer was fourth commonest cause of cancer death in females after malignancies of the breast, haematopoietic organs and liver. Based on data collected elsewhere in sub-Saharan Africa, the WHO projected that ICC was the leading cause of

female mortality among the cancers with over 2,000 deaths in the year 2010 alone (IARC,

2008; WHO/ICO, 2010a). Accordingly, the International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) ranks ICC as the second most common cancer among women in Ghana after breast cancer with an estimated 3,052 new cases and 1,556 deaths in the 2012 GLOBOCAN (Ferlay *et al.*, 2012). By the year 2025, unless there is some intervention, it is projected that there will be 5,007 new cases and nearly 3,361 cervical cancer deaths in the country annually (IARC, 2008; WHO/ICO, 2010a). Furthermore, in Kumasi, a hospital-based cancer registry has been published with data from the year 2012. Among females, the commonest cancers were cancers of the Breast (33.9%), Cervix (29.4%), Ovary (11.3%) and Endometrium (4.5%) (Laryea *et al.*, 2014)

In spite of this, the country has not got a functional population-based cancer registry or a vibrant universal screening program as is the case elsewhere and in general, very limited information about the disease, and the epidemiology of its known causative viruses can be accessed.

Cervical cancer is the end-stage of a sequence of clinically defined lesions that occur in the cervical epithelium. The spectrum of precursor cervical squamous cell abnormalities detected microscopically begins with mild dysplasia known as low-grade squamous intraepithelial lesions (LSIL) according to the Bethesda System for reporting cervical cytology corresponding to cervical intraepithelial neoplasia (CIN) 1 (Solomon *et al.*, 2002). The earliest morphologic change corresponding to this category, mild dysplasia of the epithelial lining of the cervix, is essentially undetectable by the woman. Cellular changes associated with HPV infection, such as koilocytes, are also commonly seen. LSIL may progress into high-grade squamous intraepithelial lesions (HSIL) characterized by moderate and severe dysplasia. This stage corresponds to CIN 2 and CIN 3 or carcinoma *in situ* (El-Ghobashy *et al.*, 2005). Endocervical adenocarcinoma *in situ* (AIS) is the term

for cervical glandular cell dysplasia and is a recognized precursor to endocervical adenocarcinoma (Solomon *et al.,* 2002). Finally, further progression results in invasive cervical cancer (squamous cell carcinoma or adenocarcinoma). In principle, these squamous and glandular precursor lesions are reversible and may or may not proceed to invasive cervical cancer. However, the higher the grade of the lesion, the more likely progression to cancer becomes (Horrigan and Herrington, 2006). Progression to invasive disease may take several years. Knowledge of this sequence of events in the development of invasive cervical cancer has been the basis of many cervical smear cytology screening programmes worldwide. As a result, the Papanicolau test has become a longstanding primary screening tool for CIN and invasive cervical cancer, particularly of squamous type (Horrigan and Herrington, 2006).

It is a well-established fact that, persistent infection with oncogenic (or high-risk) human papillomavirus (HPV) is an important contributor for the development of ICC and precursor lesions (Kjaer *et al.,* 1996; Walboomers *et al.*, 1999). Nearly all cases of cervical cancer arise from human papillomavirus infection that leads to squamous intraepithelial lesions/ cervical intraepithelial neoplasia (SIL/CIN) (De Villiers *et al.*, 2004). In fact, HPV can be detected in the vast majority of ICC specimens and corresponds to the highest attributable fraction as a causative agent for any major human cancer worldwide (Walboomers *et al.,* 1999). The overall prevalence of HPV in women with ICC has been reported to be as high as 99.7% around the world (Walboomers *et al.*, 1999).

Papillomaviruses are small, non-enveloped, highly epitheliotropic, double-stranded DNA viruses that infect mucosal and cutaneous epithelia in a wide variety of higher vertebrates in a species-specific manner and induce cellular proliferation (IARC, 2007). Many of these HPV types have been shown to be ubiquitous and distributed globally (Cogliano *et al.*,

2005). The genomes of all HPV types contain approximately eight open reading frames (ORFs) that are all transcribed from a single DNA strand (IARC, 2007). The ORF is divided into three functional parts: the early (E) region that encodes proteins (E1–E7) necessary for viral replication; the late (L) region that encodes the structural proteins (L1 and L2) that are required for virion assembly and a largely non-coding part that is referred to as the long control region (LCR) or upstream regulatory region (URR) (IARC, 2007). The L1 ORF is the most conserved region within the genome and has therefore been used for the identification of new papillomavirus types over the past 20 years according to an internationally agreed convention endorsed by the International Committee on the Taxonomy of Viruses (ICTV) (De Villiers *et al.*, 2004).

A new papillomavirus isolate is recognized if the complete genome has been cloned and the

DNA sequence of the L1 ORF differs by more than 10% from the closest known type. Differences in homology of between 2% and 10% define a subtype and those of less than 2% define a variant (Cogliano *et al.*, 2005). Presently, over 170 HPV subtypes are known (de Villiers, 2013) and more than one hundred human and animal papillomavirus genotypes (types) have been completely sequenced (Zheng and Baker, 2006). Out of this number, approximately 40 different HPV genotypes have been detected in the anogenital mucosa (Zur Hausen, 1996b) and at least 18 of these viruses have been associated with cervical cancer (Munoz *et al.*, 2003a; De Villiers *et al.*, 2004).

On the basis of their epidemiological association with the development of cervical carcinoma, a group of so-called high-risk HPV genotypes has been defined. These include HPV genotype 16 (HPV-16), HPV-18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68 (Bosch *et al.*, 1995a; Cuzick *et al.*, 1996; zur Hausen, 1996a; Jacobs *et al.*, 1997).

Of this high-risk group, HPV-16 and HPV-18 are the most frequently reported HPV types, causing approximately 70% of ICC cases worldwide (Munoz *et al.*, 2003b; Arbyn *et al.*, 2011). Other genotypes, such as HPV-6, -11, -42, -43, and -44, are rarely associated with cervical carcinoma and are classified as low-risk types and cause benign hyperplastic proliferations of the epithelium called warts (Lorincz *et al.*, 1992).

Detection of high-risk HPV infections is used to identify women who are at increased risk of development or progression of a cervical lesion (Cox *et al.*, 1995; Cuzick *et al.*, 1995), and conversely, negative tests have a very high negative predictive value for the development of a cervical lesion (Khan *et al.*, 2005; Wright *et al.*, 2015). However, not every single infection with HPV automatically results in cancer. In any infection, there are two possible outcomes: the infection may either resolve or persist and progress undetected to cancer via precursor lesions (SIL/CIN). More than 90% of new human papillomavirus infections regress in 6–18 months irrespective of the age of the woman (Castle *et al.*, 2009), depending on genetic, viral or other population factors that need to be properly elucidated (Schlecht *et al.*, 2001; Trottier *et al.*, 2006; Bosch *et al.*, 2008; Wang *et al.*, 2010). Nonviral 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 play an important role in the secondary prevention of ICC, thereby reducing HPVassociated mortality (WHO/ICO, 2010b). However, due to a lack of effective screening programs in lower 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. Nevertheless, recent molecular biological techniques such as HPV-

DNA testing, have been found to be effective HPV screening methods and may facilitate early detection of ICC in developing regions (Sotlar *et al.*, 2004).

A number of PCR-based assays for the identification of the various HPV genotypes have been described. Type-specific PCR primer sets allow the identification of individual genotypes (Baay *et al.*, 1996). However, they require the performance of multiple parallel amplifications from each sample and have only been described for a limited number of HPV genotypes. Alternatively, PCR assays utilizing consensus or general primers, e.g., GP5+-GP6+, MY09-MY11, PGMY, and SPF10, allow the amplification of a broad spectrum of HPV genotypes in a single reaction (Manos *et al.*, 1989b; de Roda Husman *et al.*, 1995; Kleter *et al.*, 1998a; Gravitt *et al.*, 2000). The use of MY09-MY11 and either GP5-GP6 or GP5+-GP6+ primers in a nested PCR assay has been shown to increase the overall sensitivity compared to that of each primer pair alone (Evander *et al.*, 1992; Gravitt *et al.*, 2000). In this study, a highly sensitive and specific PCR assay based on primers that target viral E6/E7 oncogenes to distinguish individual HPV genotypes is utilized with several advantages over previous methods (Sotlar *et al.*, 2004). In this assay, consensus primers for first-round amplification of a broad spectrum of mucosal HPV genotypes, including all high-risk HPV genotypes, are combined with type-specific primers for nested PCR amplifications. In order to reduce the number of nested PCRs these primers are used in multiplex primer cocktails. This strategy allows (i) HPV genotyping based on PCR product size, (ii) extension of the assay with multiplex primer cocktails for additional HPV genotypes, and (iii) direct detection of the viral oncogenes by routine agarose gel electrophoresis (Sotlar *et al.*, 2004).

Prophylactic vaccination represents a potential primary prevention measure against ICC (WHO/ICO, 2010b; Arbyn *et al.*, 2011). Three prophylactic vaccines containing virus-like particles that offer protection against cervical pre-cancers and cancers are available.

Initially, a bivalent vaccine (Cervarix™ [GlaxoSmithKline, Belgium]) containing virus-like particles for HPV-16 and -18 and a quadrivalent vaccine (Gardasil™ [Merck and Co., Inc, Whitehouse Station, New Jersey, USA]) containing virus-like particles for HPV-6, -11, -16 and -18 (Lu *et al.*, 2011) were approved for commercial sale. Much recently, during its February 2015 meeting, the Advisory Committee on Immunization Practices (ACIP) recommended an FDA approved nonavalent (9-valent) human papillomavirus (HPV) vaccine (9vHPV) (Gardasil 9, Merck and Co., Inc.) containing HPV 31, 33, 45, 52, and 58 VLPs in addition to the quadrivalent vaccine coverage (FDA, 2014 ; Petrosky *et al.*, 2015). 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 women (Harper *et al.*, 2004; Lu *et al.*, 2011; Petrosky *et al.*, 2015). Additionally, the quadrivalent and nonavalent vaccines targeted against HPV 6, and 11 can prevent 90% of all cases of genital warts (Villa *et al.*, 2005; Petrosky *et al.*, 2015). Although the bivalent and quadrivalent vaccines have been licensed in Ghana and are available in the private sector, they have not as yet been included in the national immunization program (MOH, 2014). The largest national immunization of girls in Ghana was the reported immunization of approximately 6000 school girls in fourth grade (age 9) as well as out of school girls aged 9 to 11 in four districts in the Northern and Greater Accra regions (GAVI, 2014).

In sum, current data on the prevalence of HPV infection and its type-distribution in cervical cancer are limited in Ghana and previous studies in Ghana have characterized the HPV infection and type-distribution among women and archived histological samples with a

diagnosis of ICC and cervical intraepithelial neoplasia 2/3 (CIN 2/3) (Domfeh *et al.*, 2008; Attoh *et al.*, 2010; Denny *et al.*, 2014). Since the distribution of HPV genotypes in various geographical areas and populations varies widely (Bosch *et al.*, 1995c; Clifford *et al.*, 2005a; Clifford *et al.*, 2005c), it is important to delineate the prevalence and distribution of different HPV types found in the unscreened population of women and to understand the oncogenic function of each genotype in the general population. Also, in developing countries like Ghana, on the brink of launching effective vaccination exercises on a meaningful scale, population-specific data is required for HPV type replacement monitoring in the future evaluation of deployed vaccines. Therefore, this study was undertaken with the primary objective of assessing the prevalence of HPV-16, HPV-18 and other oncogenic HPV types among the general population of unscreened Ghanaian women. Such data is critical for planning population-based interventions and especially for monitoring the impact of vaccination in the country.

1.2 HYPOTHESIS

The diagnostic frequency of cervical cancer and cervical precancerous lesions among previously unscreened women presenting for screening in Kumasi will be different from women from more developed countries where there are well-established cervical screening programs. Also, the prevalence of human papillomavirus genotypes in the population is expected to be high owing to the absence of primary prevention/ universal vaccination of adolescent girls as is the case in well-resourced settings and the detection frequencies of individual HPV genotypes in the population should differ from established patterns in women with clinically diagnosed cervical cancer in order to reflect the variation in oncogenic potential of the different genotypes.

1.3 PROBLEM STATEMENT

Cervical cancer is the commonest gynaecologic cancer in Ghana. Already, cervical cancer accounts for 13% of female cancers in developing countries (IARC, 2008) representing 80 to 86% of the total global burden of the disease owing to the lack of much needed screening and treatment facilities and limited health insurance coverage (Parkin and Bray, 2006; WHO/ICO, 2010a). Unfortunately, very little is known of the epidemiology of the virus in Ghana. In fact many existing policies on cervical cancer prevention are based on studies from other developing countries. However, local differences in the distribution of HPV genotypes may exist and need to be evaluated since they have important ramifications for monitoring and evaluating preventive vaccination programs. For population-based preventive programs to be effective and successful, the epidemiology and natural history of HPV infection in Ghana must be well understood in order to inform both policy makers and health care providers.

Since HPV infection does not cause cervical cancer in isolation, other cofactors necessary for progression from cervical HPV infection to cancer need to be assessed in the population (Munoz *et al.*, 1993). Tobacco smoking (Kjellberg *et al.*, 2000), high parity (Almonte *et al.*, 2011; Jensen *et al.*, 2011), long-term hormonal contraceptive use (Munoz *et al.*, 2006), pregnancy and co-infection with HIV, Chlamydia trachomatis (Schacter *et al.*, 1982) have been identified as established cofactors. Chemotherapy and malnutrition also lead to immunosuppression and are probable cofactors (Luque *et al.*, 1999; Temmerman *et al.*, 1999). Genetic and viral factors apart from the infecting genotype, such as molecular variants of type and viral load, are also significant (Munoz *et al.*, 2003a).

1.4 JUSTIFICATION

Cervical cancer is a great public health problem globally and is caused by persistent infection with oncogenic human papillomaviruses. In Kumasi, recent data from the cancer registry at the Komfo Anokye Teaching Hospital indicates the incidence of cervical cancer is high. It is the second leading cause of cancer after breast cancer in the Region. As a result of this, the prevalence of HPV infection is also thought to be high. In populations at high risk of HPV infection as a result of low screening and vaccination coverage, the diversity of prevalent HPV genotypes and the high incidence of multiple infections make it necessary to develop and demonstrate reliable methods for the accurate identification of the various HPV genotypes, not only for epidemiologic studies but also for clinical management of patients.

PCR-based assays for the identification of various HPV genotypes have been in existence for some time now. In these methods, PCR assays utilizing consensus or general primers, e.g., GP5+-GP6+, MY09-MY11, PGMY, and SPF10, allow the amplification of a broad spectrum of HPV genotypes in a single reaction (Manos *et al.*, 1989b; de Roda Husman *et al.*, 1995; Kleter *et al.*, 1998a; Gravitt *et al.*, 2000). Subsequently, general primer-mediated amplification products can be analyzed by various methods, e.g., direct sequencing, restriction fragment length polymorphism analysis, and hybridization with type specific probes (Chan *et al.*, 1995; Gravitt *et al.*, 1998; Sasagawa *et al.*, 2000). Most of these HPV detecting methods utilize highly conserved regions of the viral L1 major capsid gene as the target for their primers and therefore have limited sensitivity. In a persistent HPV infection, parts of the L1 region may be deleted during viral integration (Schwarz *et al.*, 1985; Choo *et al.*, 1988), making E6/E7-based consensus primers more ideal than routine methods targeting the more productive L1 region (Husnjak *et al.*, 2000). Sotlar et al., 2004 reported

a novel PCR assay with the viral E6/E7 oncogenes as the primer target region. In this assay, consensus primers for first-round amplification of a broad spectrum of mucosal HPV genotypes, including all high-risk HPV genotypes, were combined with type-specific primers for nested PCR amplifications. This strategy allows greater sensitivity and specificity than L1-based assays and allows direct detection of the viral oncogenes. Using this strategy in the present study will give reliable data on the type-specific distribution of human papillomavirus (HPV) genotypes in Kumasi for the first time.

In the context of confirmed cervical cancer cases, HPV-16 and -18 are the commonest HR HPV types worldwide. Available data from studies outside the country indicate that these two alone contribute to over 70% of all cervical cancer cases, between 41% and 67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions around the globe (Bosch *et al.*, 2008). After HPV-16/18, the six most common HR HPV types namely 31, 33, 35, 45, 52 and 58 are estimated to be the same in all world regions, although proportions differ (Clifford *et al.*, 2006a). These account for an additional 20% of cervical cancers worldwide (Clifford *et al.*, 2006a). In a post-vaccine era, such dominant high-risk HPV types unaccounted for directly by available vaccines may become more significant protagonists of cervical abnormalities. Therefore while cohort studies among cervical cancer patients and prospective studies on archival histological specimen are useful for providing immediate attributable cancer risk from various HPVs, population based studies such as this are needed to provide estimates of the potential level of risk that could emerge in such an era in the foreseeable future.

Data on the epidemiology of HPV infection in the unscreened, female population in Ghana is very limited and most previous studies have concentrated on the capital, Accra (Wiredu and Armah, 2006; Domfeh *et al.*, 2008; Attoh *et al.*, 2010; Denny *et al.*, 2014). Presently,

there is no published data on the molecular epidemiology of HPV in Kumasi, the second most populated city in Ghana after Accra and capital of the Ashanti Region. The attribution of individual HPV types to cervical intraepithelial lesions varies ethno-geographically (Vaccarella *et al.*, 2006). Even the proportion of women infected with HPV varies greatly across populations as a result of local variation in risk factors associated with genital HPV infection and persistence between populations (Trottier and Franco, 2006) and can affect the effectiveness of primary prevention programs particularly in HIV-endemic populations. Several variables like genetic variation in human leukocyte antigen (HLA) types and sociodemographic factors such as sexual behaviour and age have already been found to result in differential susceptibility to HPV infection (Castle *et al.*, 2002; Munoz *et al.*, 2006) particularly in areas plagued by HIV/AIDS. Since the profile of these risk factors differ from one population to another, the epidemiology of human papillomaviruses have been found to differ from one region to another as well. As such, population-based HPV genotyping studies allow us to establish and compare HPV genotype distributions in populations with different risk factor profiles, to investigate associations with cervical precancerous and (to a lesser extent cancerous lesions) and to appreciate the behaviour of individual HPV genotypes in natural infection.

Furthermore, HPV typing is of importance for characterizing study populations and for monitoring the efficiency of HPV-targeted therapies and vaccines (van den Brule *et al.*, 2002). Issues related to the epidemiology of HPV infection need to be properly ascertained in Ghana in order to provide the basis for follow-up studies when vaccination against HPV infection using one or all of the available vaccines is universally rolled out in Ghana as a national strategy for cervical cancer prevention, and to determine the specific sociodemographic groups that would need to be aggressively targeted for vaccination to

obtain optimum protection. Reliable knowledge of the type-specific distribution of HPV along the spectrum of cervical abnormalities will allow us to estimate the total burden attributable to vaccine preventable HPV types after taking into account the extended cross-protection offered by available vaccines and by extension the impact 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). In a developing country like Ghana, seeking to launch an effective vaccination exercise on a meaningful scale, a population-specific data would be required for vaccine cost-effectiveness assessment and type replacement monitoring.

Thus the paucity of reliable literature 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 Ashanti 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 AIM OF STUDY

The overall aim of the study was to determine the distribution of HPV genotypes using a nested multiplex PCR (NMPCR) assay that combines degenerate viral E6/E7 consensus primers and type-specific primers for the detection and typing of human papillomavirus (HPV) genotypes 6/11, 16, 18, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 56, 58, 59, 66, and 68

in previously unscreened Ghanaian women in the Kumasi Metropolis. The characteristics of Ghanaian women with cervical HPV infection in the Kumasi Metropolis will also be described as part of a general effort to study the epidemiology of HPV infection in Ghana.

1.5.1 SPECIFIC OBJECTIVES

Specific Objective 1: By means of the Papanicolau smear/test, the study estimated the prevalence of cervical cancer and epithelial cell abnormalities among women in the Kumasi Metropolis, Ashanti Region, Ghana.

Specific Objective 2: Using a highly sensitive nested multiplex PCR assay, the study estimated the prevalence of HPV E6/E7 oncogenes among women in the Kumasi Metropolis, Ashanti Region, Ghana

Specific Objective 3: The study investigated the prevalence and distribution of HPV genotypes in cervical disease by cross-tabulating individual cases according to HPV and cytology results

Specific Objective 4: The study investigated the extent of multiple human papillomavirus (HPV) infections, and which combinations of high-risk types, have the tendency to cluster together by means of inferential statistics.

Specific Objective 5: The study investigated the correlates of genital HPV infection and abnormal cytology and their implications for disease prevention using logistic regression methods.

Chapter 2 LITERATURE SURVEY

2.1 MOLECULAR BIOLOGY OF HUMAN PAPILLOMAVIRUSES (HPV)

The papillomaviruses are a ubiquitous group of viruses known to infect mammals, birds and reptiles, with species- and tissue-specificity (Bosch *et al.*, 2006). They are one of the oldest, largest, and most diverse of the known virus families. Human papillomaviruses (HPVs), like all papillomaviruses, target the stratified squamous epithelia of the body (Manhart and Koutsky, 2002). A subset is also able to infect the glandular epithelium of the cervix (Bosch *et al.*, 2006).

2.1.1 Structure and Components of Human Papillomaviruses

Papillomaviruses are small, rounded, non-enveloped DNA tumour viruses with an icosahedral virion size of ~55 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). The L1 outer protein coat contains 72 pentamers, or capsomers of L1, the major capsid protein, centred on the vertices of a $T = 7$ icosahedral lattice. The L2 protein is largely an internal protein and is present at about onethirtieth of the abundance of L1 (Stanley *et al.*, 2006). 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.

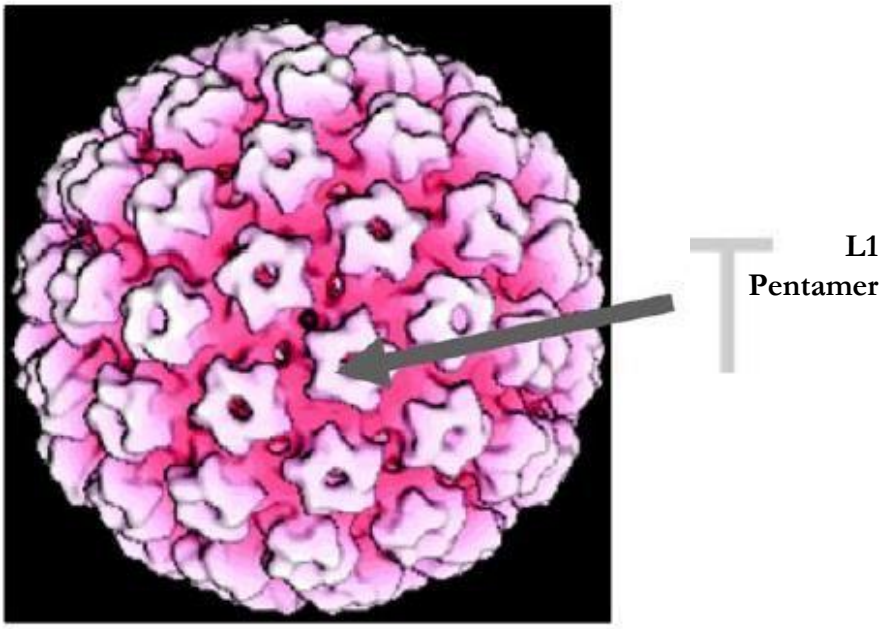

Figure 2.1: A model of the virus capsid depicting superficial pentamers. These conformational epitopes on the major coat or capsid protein L1 displayed on the outer surface of the intact virus particle can be recognized by the immune**2.1.2 Organization of the HPV Genome** system and forms the basis of HPV vaccines. Source: Stanley *et al.*, 2006.

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 (Park *et al.*, 1995). 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. 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. 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 and Baker, 2006). 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) (Danos *et al.*, 1982) 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 (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, suggesting that it may play a part in determining the range of hosts for specific 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 and code for the major (L1) and minor (L2) capsid proteins that encapsulate the viral DNA (Park *et al.*, 1995) and are useful in the setting of vaccine design (zur Hausen, 2002). Notably, the viral genome does not encode a unique DNA polymerase, which is required for DNA replication, and therefore the virus must depend upon the replication machinery of the host-cell for the generation of viral progeny (Munoz *et al.*, 1996).

2.1.3 Phylogenetic Organisation and Taxonomy

Papillomavirus genomes are thought to be very static, and sequence mutations or recombination are very rare events. Mutational changes apparently occur at frequencies that do not differ greatly from those of the DNA genomes of the infected host organism (Cogliano *et al.*, 2005).

Different genera share less than 60% nucleotide sequence identity in the L1 ORF. Species within a genus share between 60 and 70% nucleotide sequence identity. 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). The traditional papillomavirus types within a species share between 71 and 89% nucleotide sequence identity within the complete L1 ORF (De Villiers *et al.*, 2004). Over 170 HPV types have been characterized based on DNA sequence homology instead of conventional serology (de Villiers, 2013). Subtypes have between 90% and 95% homology, and variants between 96% and 98% (De Villiers *et al.*, 2004).

Phylogenetically, papillomaviruses are officially recognized by the International Committee on the Taxonomy of Viruses (ICTV) to belong to the family Papillomaviridae having 18 genera (Fig. 2). The approximately 40 types able to infect the human ano-genital tract (De

Villiers et al., 2004), collectively called the genital HPVs, belong to the alpha genus (Fig. 3). 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)

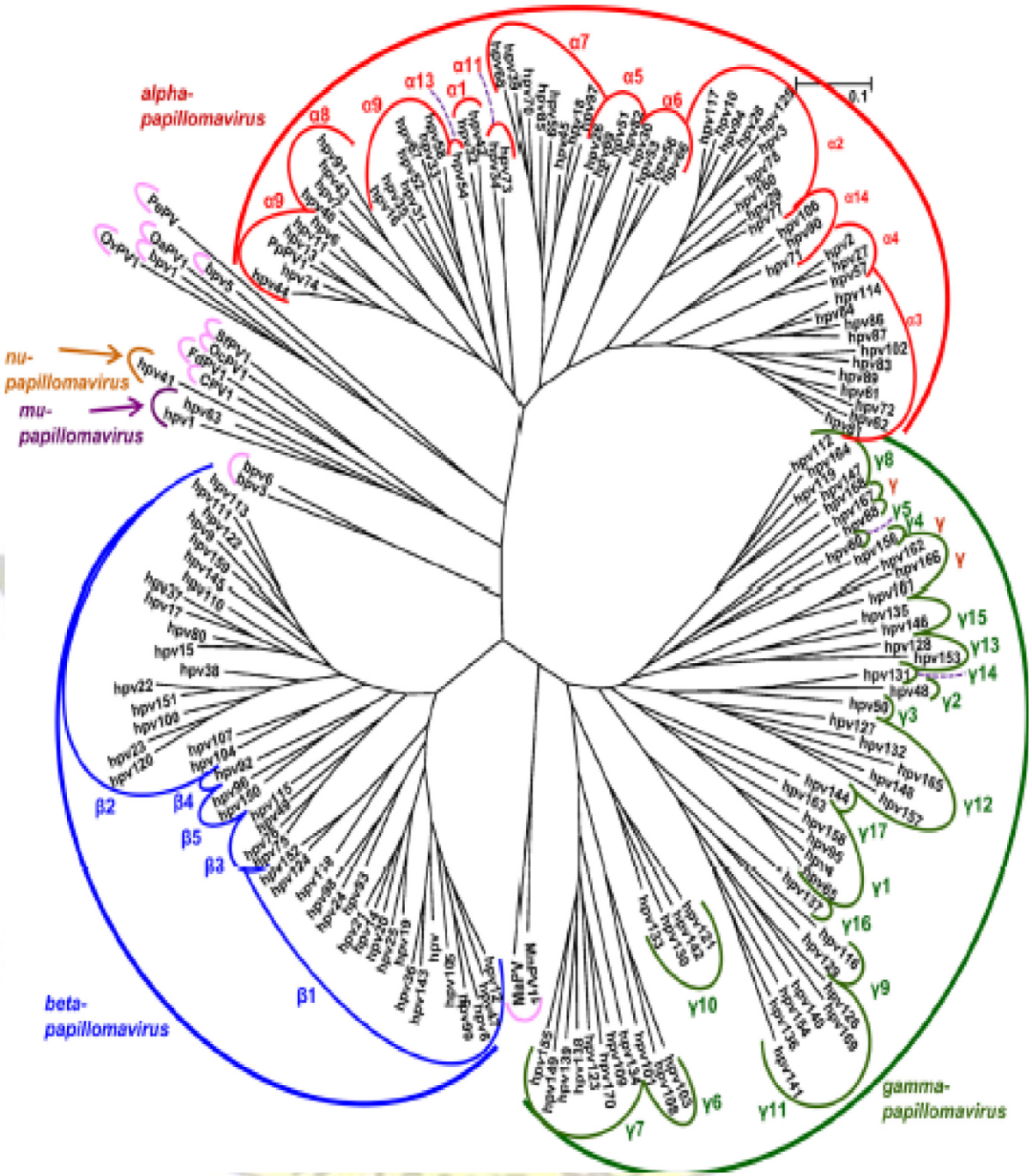

Figure 2.2: Phylogenetic analysis based on the L1 ORF sequences of 170 HPV types, as well as single animal papillomaviruses, using the maximum likelihood method. The tree was constructed using the MEGA5.1 program. ***Source***: de Villiers, 2013.

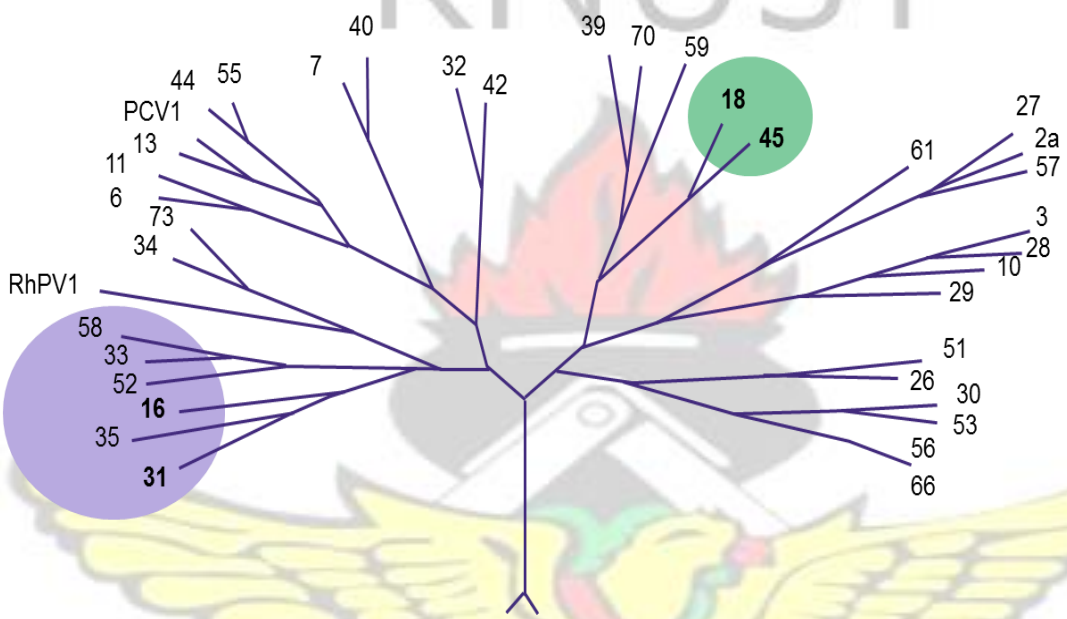

Figure 2.3: The alpha-papillomavirus genus of the papillomavirus phylogenetic tree. Oncogenic types closely related to HPV 16 and 18 are highlighted HPV 16 is most closely related to HPV 31. HPV 18 is most closely related to HPV 45. ***Source:*** Rose and Stoler, (2006).

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2.1.3.1 Classification of HPV genotypes

Although not completely reflective of phylogeny, it is convenient to classify HPVs into three groups according to oncogenic potential and associated diseases. Three classes of HPV are recognised, namely: high-, intermediate- and low-risk HPV (Munoz *et al.*, 2003a). Phylogenetic relationships alone cannot predict risk status, and the essential criterion for

such classification remains the predominant disease association of the HPV type in question (Lacey *et al.*, 2006). 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. E6 and E7 from low-risk HPVs, inactivate cellular p53 and pRb tumor suppressor proteins less efficiently than do E6 and E7 from high-risk HPVs (Münger and Howley, 2002).

2.2 DISEASES ASSOCIATED WITH HPV

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, These are;

(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).

2.2.1 Benign HPV-associated cervical lesions

A cervical wart or condyloma results from the infection of the cervix with low-risk HPV types. It may be diagnosed by cytologic evaluation of a Papanicolaou (Pap) smear or at the time of colposcopic evaluation for an abnormal Pap smear. Rare papillary-type cervical warts are easy to see because they are exophytic. In contrast, a flat cervical condyloma may not be clinically evident on gross inspection of the cervix at the time of routine gynaecologic examination. 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).

2.2.2 Pre-malignant HPV-associated lesions

2.2.2.1 Squamous Intraepithelial Lesions (SIL) /Cervical Intraepithelial Neoplasia (CIN)

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.*, 2004b). The pre-malignant changes characteristic of cervical cancer represent a spectrum of histological abnormalities ranging from Low grade squamous intraepithelial lesion (LSIL)/CIN1/mild dysplasia through High grade squamous intraepithelial lesion

(HSIL)/CIN2/moderate dysplasia to HSIL/CIN3/severe dysplasia/carcinoma-in-situ (Woodman *et al.*, 2007). HSILs are mostly found in association with HR-HPV while LSILs are frequently associated with LR-HPV-6 and -11 (Lacey *et al.*, 2006). A meta-analysis of 55 studies reported HPV-6 to be present in 8.1% of HPV-positive LSIL cases and HPV-11 in 3.2% of cases (Lacey *et al.*, 2006).

2.2.3 Cervical Cancer

It is now generally accepted 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.*, 2004b). Almost 90% of cervical cancers are squamous cell carcinomas (ACS, 2010),. The fraction of cervical cancer attributable to HPV-16 is 53.5% (Munoz *et al.*, 2004a). In fact it is worth noting that HPV 16 predominates in SCC while HPV-18 predominates over type-16 in the adenocarcinomas (ACS, 2010). Taken together HPV-16 and HPV-18 account for about 70% of all cervical squamous cell carcinomas. Furthermore, the estimated HPV-16/18 fraction is higher in more developed (72–77%) than in less developed (65–72%) regions. About 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 estimated to be HPV-16/18-positive (Munoz *et al.*, 2004a).

2.3 EPIDEMIOLOGY OF HPV INFECTION

Human papillomavirus infection is the commonest sexually transmitted condition contributing 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, 1996a; WHO, 1999).

Several studies have reported 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 highrisk" 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, 2003).

2.3.1 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 agestandardized 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.*, 2000a). 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).

2.3.2 Prevalence of HPV Genotypes in Cervical Cancer

A pooled analysis of 12 studies conducted in 25 countries with standardised protocol and GP5+/6+ HPV-DNA testing using PCR in a central laboratory estimated HPV type-specific prevalence in 3085 cervical cancer cases (Munoz *et al.*, 2004a). The overall HPV-DNA prevalence was 96% and the 15 most common types were, in descending order of frequency, 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 common types (HPV-16, -18, -45, 31, -33, -52, -58 and 35) accounted for 89% of all cervical cancer cases worldwide (Clifford *et al.*, 2006a). A higher than average prevalence of HPV-16 was found in Northern Africa, Europe and North America, of type 45 in sub-Saharan Africa and of type 31 in Latin America (Munoz *et al.*, 2004a). A comprehensive meta-analysis of more than 14,500 cases from studies published up to January 2006 has been conducted (Curtis and Sutherland, 2004) the findings of which corroborate strongly the results of the pooled study. The most

common HPV types identified were, in order of decreasing prevalence, HPV-16, -18, -31, 33, -35,-45, -52, and -58; with the slight possible exception of HPV-56 being the eighth most common type instead of HPV-52 in Europe. HPV-16 prevalence varies from 52% in Asia to 58% in Europe, and HPV-18 prevalence varies from 13% in South/Central America to 22% in North America (Curtis and Sutherland, 2004).

2.3.3 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.*, 2005a), 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.*, 2004a). 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).

2.3.4 Transmission of HPV

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).

2.3.5 Natural History of Cervical HPV Infection

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).

2.3.5.1 Multiple Infections

Co-infection with multiple HPV types is found in 20% to 50% of HPV-infected women (Liaw *et al.*, 2001b; 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.*, 2000b; Liaw *et al.*, 2001b; Rousseau *et al.*, 2001b; 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 suggest that the presence of multiple types does not especially influence persistence of HPV infections (Molano *et al.*, 2003; Plummer *et al.*, 2007). It is held 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.*, 2000b; Mendez *et al.*, 2005).

2.3.6 Burden of HPV-Related Diseases

Globally HPV is responsible for 5% of all human cancer, over 10% of cancer in women and up to 15% of cancer in women in developing countries (Winer and Koutsky, 2004).

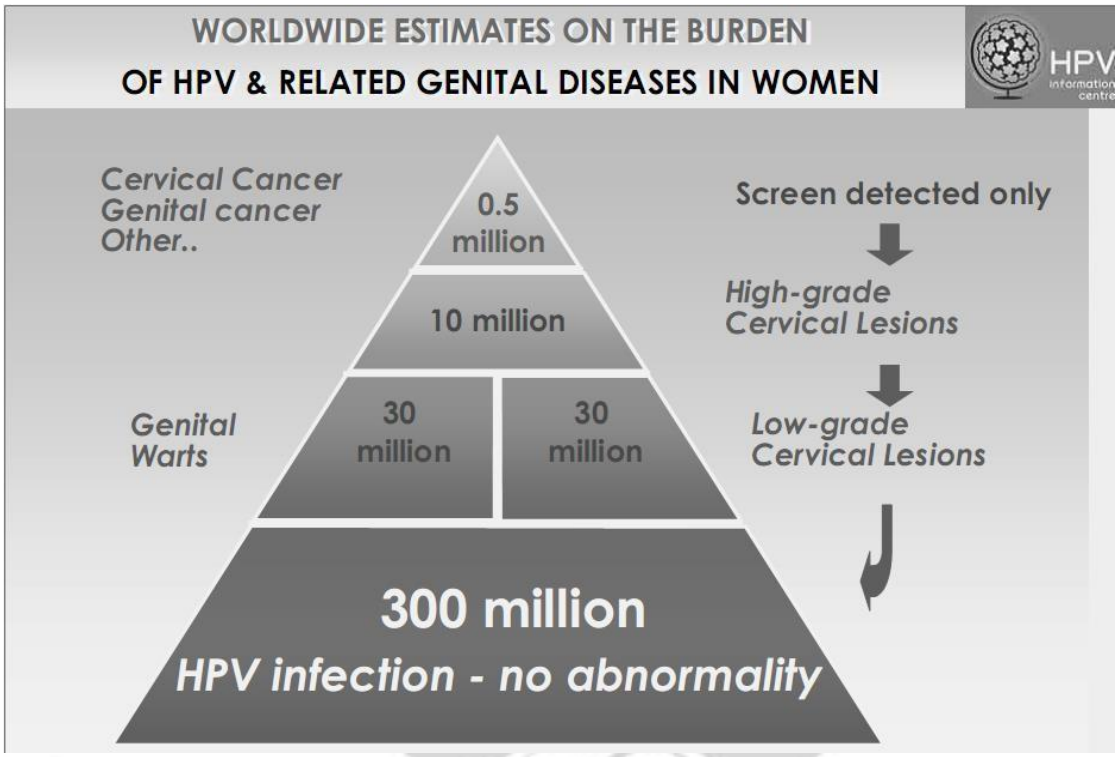

Figure 2.4: Estimates of the global burden of HPV related diseases in women. The worldwide number of cases, rounded for convenience. Adapted from (WHO/ICO, 2010a).

2.3.7 Burden of Cervical Cancer

2.3.7.1 Africa and the World: HPV-related Burden of Cervical Cancer World-wide, cervical cancer is the second commonest cause of female cancer after breast cancer. About half a million cases of cervical cancer are diagnosed each year, with 275,000 deaths occurring. Eighty-six percent of cases occur in *developing countries* where 2 out of every 100 women are likely to get it before age 65 (Parkin and Bray, 2006; WHO/ICO, 2010a). This largely owes to the lack of much needed primary screening, treatment and health insurance coverage in 'developing countries' (WHO/ICO.2010a). In the so called *developed countries* less than 0.5% of women will develop cervical cancer before age 65.

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 (WHO/ICO, 2010a).

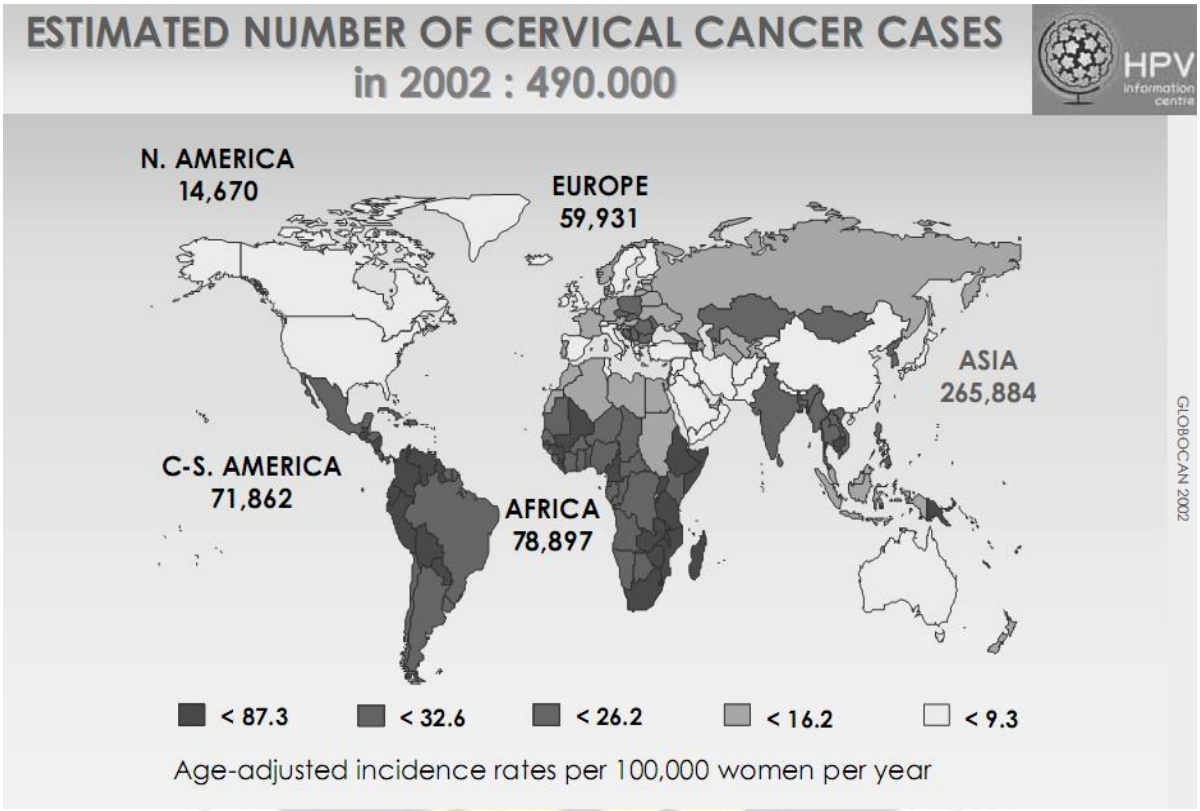

Figure 2.5: Global estimates of cervical cancer incidence. The highest adjusted incidence rates occur in Sub -Saharan Africa where fewer studies have been carried out compared to most of Europe and America which have recorded the least number of cases. Adapted from (WHO/ICO, 2010a).

Mortality rates are substantially lower than incidence. Worldwide, the ratio of mortality to incidence is 55% (Parkin and Bray, 2006). Because cervical cancer affects relatively young women, it is an important cause of lost years of life (YLL). In Latin America, the Caribbean

and Eastern Europe, cervical cancer makes a greater contribution to YLL than diseases such as tuberculosis, maternal conditions or AIDS. It also makes the largest contribution to YLL from cancer in the populous regions of sub-Saharan Africa and South-Central Asia (Parkin and Bray, 2006).

2.3.7.2 Ghana: Burden of HPV-related Cervical Cancer

Ghana has a population of 6.57 million women aged 15 years and older who are in the age group at risk of developing 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 10-year review of autopsies and hospital mortality in which cervical cancer was fourth commonest cause of cancer death 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, 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 commonest cancers were cancers of the Breast (33.9%),

Cervix (29.4%), Ovary (11.3%) and Endometrium (4.5%) (Laryea *et al.*, 2014).

2.4 MORPHOGENESIS OF HPV INFECTION

2.4.1 Cytological Features of HPV Infection

HPV infection leads to squamous intraepithelial lesions (SIL) and subsequently to cervical cancer (Figure 6) with well-defined morphologic hallmarks. Dysplastic changes, including increased nuclear basophilia, mitotic figures, high nuclear/cytoplasmic (N/C) ratio, and disorganized growth are observed at different levels in the mucosa (Robboy *et al.*, 2008).and can be used to prognosticate the extent of disease. Both oncogenic and nononcogenic strains of HPV have the capacity to induce cytologic changes (Feichter and Meisels, 2002) which can be detected by Papanicolaou staining (Syrajnen *et al.*, 1987).

In low-grade squamous intraepithelial lesions (LSIL), the virus proliferates as an episomal moiety to produce millions of HPV viral copies that accumulate in the cell cytoplasm and can be seen microscopically as koilocytes (ASCCP, 2012). The koilocyte is a classical feature of HPV infection where intermediate and superficial cells show perinuclear halo with eccentric single or binucleated hyperchromatic nuclei with mild irregularity of outline (Figure 6A) (Kashyap *et al.*, 2011). Thus koilocytes are characteristic of the last stage of viral replication and the egress of virions and are regarded as the most pathognomic feature of HPV-mediated LSIL with a high degree of specificity (Kashyap *et al.*, 2011).

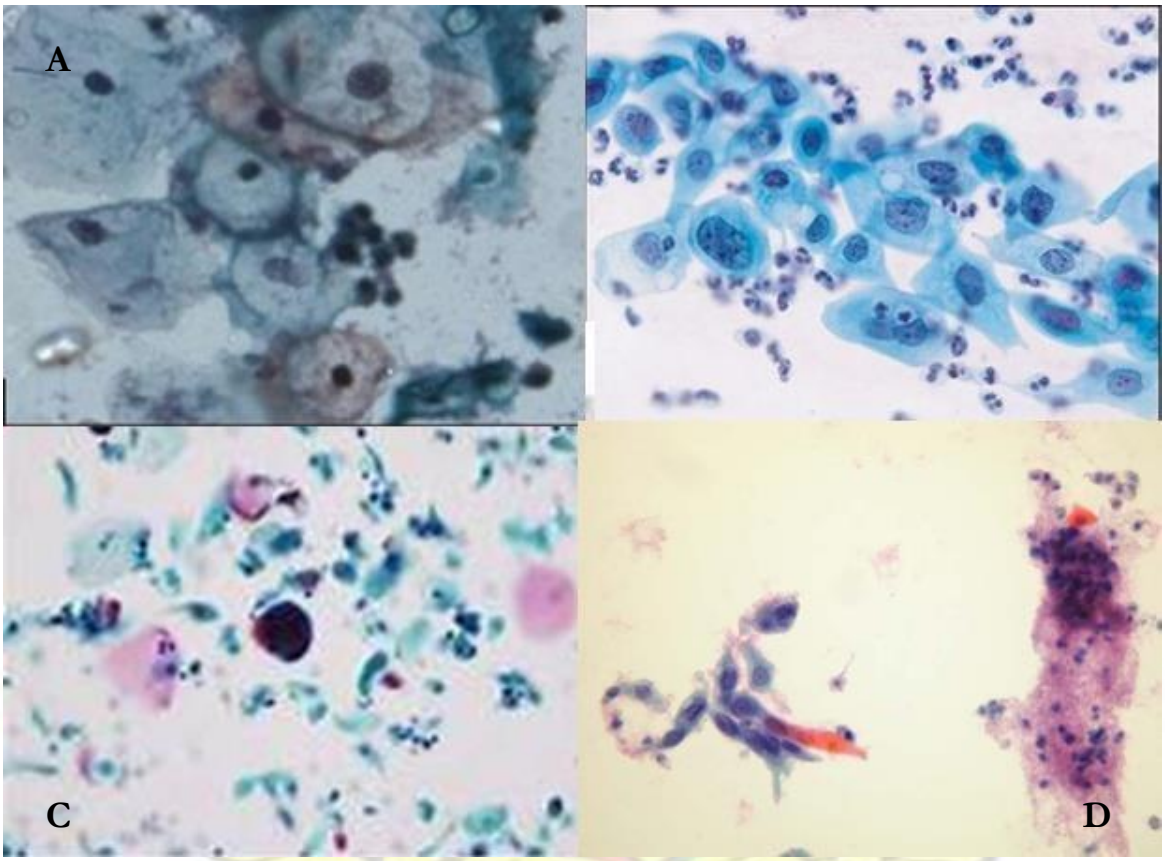

Figure 2.6: Features of abnormal cervical cytology. (A) LSIL: clustered cells demonstrating the enlarged nuclei, hyperchromasia and koilocytosis (cytoplasmic clearing) typical of an LSIL lesion. Adapted from (Kashyap *et al.*, 2011) (B) HSIL: cellular cluster showing markedly enlarged nuclei, hyperchromasia associated with coarse chromatin, and irregular nuclear membranes typical of an HSIL lesion. (C, D) Cancer (Liquid based-Papanicolaou stain x 400): Irregular cell forms; nuclei are enlarged with prominent nucleoli. The cell cluster at right shows diathesis ("cotton candy necrosis"). Adapted from: http://www.asccp.org as at 26th July, 2014.

However, not all HPV infected cells show the features of koilocytosis, especially those from dysplastic lesions (Koss and Melamed, 2005; Kashyap *et al.*, 2011). The non-classical signs such as rounding of cell margin, mild nuclear hyperchromasia and small, pyknotic, eccentric nuclear might be adequate, though less specific, indicators of HPV infection as well (Kashyap *et al.*, 2011).

Severely dysplastic cells categorized as HSIL display a high nuclear-to-cytoplasmic ratio and irregular nuclear membranes (ASCCP, 2012). Usually a cluster of cells exhibiting a loss of polarity, nuclear enlargement and coarsely granular chromatin together with a high nuclear to cytoplasmic ratio, warrant a diagnosis of HSIL. Some nuclei display discrete nucleoli (ASCCP, 2012).

2.5 PATHOGENESIS OF HPV INFECTION

Papillomaviruses infect basal cells of the host and proliferate in the differentiating epithelia by exploiting the host's cellular machinery to their own advantage (Lowy and Howley, 2001). The virus gains access to the basal cells of the cervix through micro-abrasions in the cervical epithelium such as occurs during penetrative sex. Infectious virions are then able to reach the basal layer of the epithelium, where they bind to and enter into cells thus initiating an infectious cycle (Doorbar, 2005). It has been suggested that for maintenance of the infection, the virus has to infect an epithelial stem cell (Doorbar, 2005). The replication cycle within the epithelium can be divided into two distinct events. First, the early HPV genes E1, E2, E4, E5, E6 and E7 are expressed from episomal DNA, then the viral genome is replicated and maintained at a low copy number of about 100 for varying periods of time within the infected, but still replicating competent cells under the regulation of the E1 and

E2 viral genes. E1 and E2 viral proteins are essential for this basal DNA replication. Second, once the basal cells are pushed to the suprabasal compartment, they lose their mitotic ability and instead initiate the terminal differentiation program. The papillomavirus genome replicates further to activate the late genes L1 and L2. E4 is also expressed alongside to regulate the egress of virions. L1 and L2 encapsulate the viral genomes to form

progeny virions in the nucleus that are released into the environment by taking advantage of the natural disintegration of the epithelial cells. The shed virus can then initiate a new infection (Motoyama *et al.*, 2004). Low-grade intraepithelial lesions support productive viral replication. Theoretically, since HSILs by and large do not make virus, it is a dead end for the virus. Rather, the viral oncogenes E6 and E7 are inappropriately expressed in this population of cells that retain the capacity to divide by ignoring pro-apoptotic cell cycle signals, thereby initiating and promoting abnormal cell proliferation in the suprabasal layers (Bosch *et al.*, 2006).

2.5.1 Molecular Pathogenesis of Cervical Cancer

Molecular evidence for the role of HPV in the onset of cervical abnormalities culminating in cancer came from experiments that demonstrated HPV-specific gene products (such as E6 and E7) in cervical cancer cell lines and cancer biopsies (Schwarz *et al.*, 1985),a plausible splice site on the viral DNA allowing integration of the viral genome (Durst *et al.*, 1983), the immortalization property of viral DNA (Durst *et al.*, 1987b; Pirisi *et al.*, 1987) and the encoded viral oncogenes (Munger *et al.*, 1989).

The critical molecules in the process of virus replication are the viral proteins E6 and E7. In experimental systems E6 (Werness *et al.*, 1990) and E7 (Munger *et al.*, 2004) interacted with a number of cellular proteins and were shown to induce proliferation and eventually immortalization and malignant transformation of cells. Together, E6 and E7 are responsible for the induction as well as the maintenance of the transformed phenotype of cervical cancer cells by binding with multiple cellular targets (Munger and Howley, 2002). The best characterized effects are on two tumour-suppressor genes, p53 and Retinoblastoma (Rb), which are central molecules in cell cycle control, and remarkably, are rarely mutated in

cervical cancers (Thomas *et al.*, 1999). The p53 is a housekeeping gene which recognizes DNA damage in a cell, arresting that cell in G1 phase of the cell cycle to allow for DNA repair or, if repair is not possible, to lead that cell into programmed death (Thomas *et al.*, 1999; Robboy *et al.*, 2008). Precocious S-phase would normally lead to apoptosis by the action of p53. However, in HPV-infected cells, this process is counteracted by the viral E6 protein, which binds with, and inactivates p53 protein causing its degradation through the ubiquitin pathway (Thomas *et al.*, 1999). Further, the inactivation of p53 protein by HPV E6 oncoprotein also leads to the upregulation of cyclin B, which regulates transition from G2 to M phase (Thomas *et al.*, 1999; Robboy *et al.*, 2008).

The retinoblastoma tumour-suppressor protein, pRb and its related pocket proteins pRb2/p130 and p107 (Masciullo *et al.*, 2000), regulate the cell cycle at the G1/S restriction point by complexing with and inhibiting the activity of E2F, which serves as a transcription-dependent promoter of cell cycle progression (Munger *et al.*, 2004; Robboy *et al.*, 2008). Binding of E7 to pRb activates the E2F transcription factor, which triggers the transcription of cyclin E and expression of other proteins necessary for DNA replication (Munger *et al.*, 2004) and thus inappropriately forces the cell past the G1/S point into S phase (Dyson *et al.*, 1989; Lavia *et al.*, 2003). As a consequence, the regulation of mitosis is compromised and normal keratinocyte differentiation is retarded (Doorbar, 2005). In addition to inactivating the pro-apoptotic protein BAK34 (Thomas and Banks, 1998), which results in resistance to apoptosis and an increase in chromosomal instability, HPV E6 can activate the telomere lengthening enzyme telomerase independent of p53 binding (CIA, 2010). E7 can induce abnormal centrosome duplication through a mechanism independent of inactivation of pRb and its family of proteins. By inducing centriole amplification, E7 also induces aneuploidy of the E7-expressing cells, which contributes to

tumourigenesis (Duensing *et al.*, 2001). Both E6 and E7 gene products can independently immortalize human cells, but only at moderate efficiency; together, they markedly increase transforming activity in an interesting complementary and synergistic manner (Band *et al.*, 1990; Halbert *et al.*, 1991).

There are differences between the E6/E7 protein expression patterns of high-risk and lowrisk HPV infection, but these are often of a quantitative rather than a qualitative nature (Longworth and Laimins, 2004). This observation and the outstanding ability of HPV-16 to persist and induce progression towards malignancy in the differentiating keratinocyte (Hawley-Nelson *et al.*, 1989) highlights the essential transforming role of the E6/E7 viral oncogenes in allowing the so-called high-risk viruses to replicate in differentiating epithelial cells that would have normally withdrawn from the cell division cycle by the action of pro-apoptotic factors (Munoz *et al.*, 1996). Another feature of high-risk HPV infection is a propensity to integrate into the host genome at this terminal stage of its life cycle (Durst *et al.*, 1987a). At first, the main effect of viral integration seems to be the loss of E2 gene sequence resulting from a nick in the episomal genome (Choo *et al.*, 1988). However, integration of HPV DNA into a host genome has been shown to positively correlate with the severity of lesions and unveils the role of E2 (Park *et al.*, 1995). Actually, it is widely believed that during tumour progression, there is constant erratic replication of the viral proteins E6 and E7 as a result of integration-related loss of E2-mediated regulation of transcription (Munoz *et al.*, 2006; Woodman *et al.*, 2007). Uncontrolled E6/E7 protein expression leads to increasing genomic instability, accumulation of oncogenic mutations, further loss of cell-growth control, and ultimately cancer (Duensing and Munger, 2004). In addition, a constant level of E6/E7 proteins is maintained via stabilization of the mRNA by the influence of modified chromatin structures as well (Bernard, 2002).

2.6 MOLECULAR MARKERS IN TESTING FOR HPV 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.

2.6.1 Pooled HPV Testing Methods

Several studies have shown that testing for HPV DNA is significantly more sensitive than Pap smears for the detection of high-grade cervical intraepithelial neoplasia (CIN 2+), SCC, adenocarcinoma in-situ (AIS) and adenocarcinoma (Bosch, 2003; Bulkmans *et al.*, 2007b; Anttila *et al.*, 2010) but it is comparatively less specific, especially in women under 30 years, largely due to the prevalence of transient, clinically benign infection (Bosch and de Sanjose, 2007). However, when put in the right age context, it is useful for primary cervical screening and triage of ASCUS (Smith *et al.*, 2002; Wright *et al.*, 2007b; Wright *et al.*, 2007a; Cuzick *et al.*, 2008).

Clinically validated HPV detection PCR assays are available that allow aggregate detection of many, if not all mucosal HPV types in one reaction and can be variously referred to as broad spectrum tests, presence/absence tests, or pooled HPV tests. These tests rely on so

called consensus primers such as GP5+-GP6+, PGMY09/11, and SPF10 directed to highly conserved regions of the HPV genome (Manos *et al.*, 1989a; Kleter *et al.*, 1998b), mostly within the L1 (Yoshikawa *et al.*, 1990; Jacobs *et al.*, 1995) and also the E6/E7 (Resnick *et al.*, 1990) regions of the HPV genome. At least in theory, such assays are potentially capable of detecting all mucosal HPV types (Bernard *et al.*, 1994). Nevertheless, despite sequence conservation, some degree of inter-type heterogeneity at the nucleotide level precludes the selection of single primer pairs that fully match corresponding sequences of a broad spectrum of HPVs. Therefore, to allow broad spectrum HPV detection, consensus primer assays either use degenerate primers with nucleotide variations at variable base positions (eg. GP5/GP6 (Snijders *et al.*, 1990) and MY09/MY11(Manos *et al.*, 1989a)),or pI-1/pI-2 primers (Novelli *et al.*, 1992) with the non-specific base-analogue inosine at ambiguous base positions, or sets of overlapping primers (multiplex) (Sotlar *et al.*, 2004). Also, parts of the L1 region may be deleted during viral integration (Schwarz *et al.*, 1985; Choo *et al.*, 1988), making E6/E7-based consensus primers more ideal than routine methods targeting the more productive L1 region (Husnjak *et al.*, 2000). In the present study, nested multiplex PCR (NMPCR) assay that combines degenerate E6/E7 consensus primers and type-specific primers was utilized for the detection and typing of human papillomavirus (HPV) genotypes.

A typical example of a commercially tailored assay is the GP5+/6+ PCR- enzyme immunoassay (EIA) or Hybrid Capture II method which has been used extensively to characterize infections by HPV types 16, 18, 52, 58 and 59 in patients with cervical cancer (Jacobs *et al.*, 1995; Jacobs *et al.*, 1997; Solomon *et al.*, 2001) and extensively validated and is recommended as a reference test for newly developed HPV tests (Klionsky *et al.*, 2008). Hybrid Capture 2™ (HC2) (Qiagen; Mississauga, Ontario, Canada) detects DNA

from 13 high-risk oncogenic types, HPV-16,-18 -31, -33, -35, -39, -45, -51, -52, -56, -58, 59, and -68 collectively through hybridization of an RNA probe cocktail with corresponding target DNA, capture of the resultant RNA-DNA hybrids with antibodies specific for the hybrids, and detection of the antibodies with a chemiluminescent substrate (Castle *et al.*, 2008).

The GP5 and GP6 primers or its second generation, extended version GP5+/6+ (de Roda Husman *et al.*, 1995), are complementary to the part of the L1 region located inside the sequence recognized by the MY09/11 PCR (Manos et al., 1989) or its modified version, PGMY09/11 (Gravitt et al., 2000) primers respectively. Therefore, they can be used either in general primer PCR or subsequent to the MY primers in a nested PCR protocol (Evander *et al.*, 1992). The MY09 and MY11 (MY) primers are degenerate primers consisting of 24 primer pairs and are able to amplify more than 25 HPVs (Manos *et al.*, 1989a).

2.6.2 Genotyping Methods

The use of MY09/MY11 and either GP5+/6+ or GP5/6 in a nested PCR has been shown to be more reliable and sensitive than each primer pair alone (Evander *et al.*, 1992; Husnjak *et al.*, 2000). As a matter of fact, the individual genotypes in a pooled PCR can always be delineated by subsequent analysis with various methods such as restriction fragment length polymorphisms analysis (Yoshikawa *et al.*, 1991), reverse line blot analysis, probe-specific hybridization as in an enzyme immunoassay (EIA) (Bauer *et al.*, 1991), direct sequencing of consensus primer PCR products (Smits *et al.*, 1992; van den Brule *et al.*, 2002), and type-specific PCR (van den Brule *et al.*, 1990). Essentially, the determination of HPV type is important owing to the different cancer-related risk of particular HPV genotypes (Lorincz *et al.*, 1992).

The nested multiplex PCR (NMPCR) assay described by Sotlar et al., (2004) which was employed in this study combines degenerate E6/E7 consensus primers and type-specific primers for the detection and typing of most high-risk human papillomavirus genotypes. It is documented that the NMPCR method is a sensitive and useful tool for HPV DNA detection, especially when exact HPV genotyping and the identification of multiple HPV infections are required (Sotlar *et al.*, 2004).

If competently performed, HPV detection tests clearly improve the detection rate of highrisk HPV DNA in women, and also avoid some false-negative diagnoses (Schlecht *et al.*, 2001); however, the assays are relatively, prohibitively expensive for a large part of the world where cervical cancer is still a principal cause of death. In addition, some specificity is lost as the tests cannot delineate between a transient and potentially transforming infection. One of the issues of type-specific testing however is the complexity of the data/result it may generate. Multiple infections are common, and, also the significance of one non–HPV-16 high-risk type over another is poorly understood (Bulkmans *et al.*, 2007a).

2.6.3 E6/E7 mRNA Detection

The oncogenic potential of the high-risk HPV genotypes is mediated by the over-expression of the two viral oncogenes, E6 and E7.The detection of E6/E7 mRNA in putative cervical epithelial cells is a sign of active transcription of these deleterious genes and is a useful marker for an increased cervical cancer risk (Poljak *et al.*, 2002; Munger *et al.*, 2004; Safaeian *et al.*, 2007). Therefore, HPV E6/E7 mRNA testing, a measure of E6/E7-mediated oncogenesis (Munger *et al.*, 2004), could be a more specific and better predictor of cervical cancer risk than HPV DNA screening. There is evidence from studies carried out on the

two commercially available E6/E7 mRNA-based assays, APTIMA® HPV assay (Dockter *et al.*,

2009; Ratnam *et al.*, 2011) and PreTect™ HPV-Proofer assay (Molden *et al.*, 2007) that E6/E7 mRNA testing may be more specific than HPV testing (Kraus *et al.*, 2006; Lie and Kristensen, 2008).

The APTIMA® HPV test (Gen-Probe Inc., San Diego, CA) is a qualitative nucleic acid amplification test that detects E6/E7 mRNA from 14 high-risk oncogenic types, HPV-16,18 -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68 collectively (Ratnam *et al.*, 2011) and is reported to have similar clinical sensitivity but significantly improved specificity to Hybrid Capture 2 (HC2), which has the same genotype panel, minus HPV -66 at detecting cervical pre-cancer and cancer (Szarewski *et al.*, 2008; Dockter *et al.*, 2009; Ratnam *et al.*, 2011).

The other commercially available E6, E7, mRNA-based assay, the PreTect™ HPV-Proofer test (Proofer; Norchip, Klokkarstua, Norway), is a real-time, multiplex nucleic acid-based amplification assay (NASBA) capable of detecting a panel of five high-risk oncogenic types, HPV-16,-18 -31, -33, and -45, isothermally using molecular beacon probes (Molden *et al.*, 2007). Proofer has been around for longer and evidence of its clinical performance is available from several European studies (Kraus *et al.*, 2004; Kraus *et al.*, 2006; Naucler *et al.*, 2007; Lie and Kristensen, 2008). Briefly, it has been demonstrated to be significantly more specific than both APTIMA® and HC2, but not sensitive enough in the environment of CIN 2+ (Szarewski *et al.*, 2008; Ratnam *et al.*, 2011).

2.6.4 E6/E7 Oncoprotein Detection

One way to improve on the specificity currently obtained with HPV DNA and mRNA testing is to develop and validate biomarkers that can identify women at risk for progressive cervical disease. Both commercial and in-house assays are available for detection of cellular proteins that are over-expressed by HPV-infected, E6/E7 deregulated cells such as the cyclin-dependent kinase inhibitor 2A, (CDKN2A) also known as p16ink4A gene product, or simply p16 (von Knebel-Doeberitz and Syrjanen, 2006) and two replication associated proteins, minichromosome maintenance protein 2 (MCM2) and topoisomerase IIA (Top2A) (ProEx™ C; Becton Dickinson) (Kelly *et al.*, 2006; Dehn *et al.*, 2007). Notable among these is the widely validated immunostain of p16ink4A, CINtec® (mtm Laboratories AG). The p16 is a cellular correlate of the increased expression of oncogenic E6/E7 mRNA in HPV-transformed cells (Agoff *et al.*, 2003). Several properties of p16 make this protein a promising biomarker for HPV-related cancers: its expression is directly linked to the HPV oncogene action which is so far the clearest proof of HPV-associated cancer and may be independent of the HPV type causing the oncogenic infection, obviating the need to detect different HPV types in DNA and RNA assays (von Knebel Doeberitz *et al.*, 1992). Also, in contrast to many classic tumour markers such as Ki-67 or MYC, p16 is not associated with proliferation, but rather with senescence and cell cycle arrest (Beausejour *et al.*, 2003), and is not found expressed in normal basal cells or in other cells with proliferative capacity (Klaes *et al.*, 2001). A dual immunostain combining antibodies of Ki-67 and p16ink4A in one assay (CINtec® PLUS, mtm Laboratories AG) substantially simplifies p16 staining and minimizes inter-observer bias by standardizing the evaluation of stained slides (Denton *et al.*, 2010; Petry *et al.*, 2011).

Chapter 3 MATERIALS AND METHODS

3.1 STUDY DESIGN AND SETTING

The present study is a multi-centre cross-sectional descriptive study to estimate the prevalence of genital HPV genotypes and their distribution among an unscreened population of women in a suburb of Ghana. A sample of 500 women presenting for screening at the Cervicare Clinics of three major health centres in Kumasi, namely: the Kumasi South Regional Hospital (KSRH), the Tafo Government Hospital (TGH) and the Suntreso Government Hospital (SGH) all in Kumasi in the Ashanti Region of Ghana, during May 2012 to November 2014 were selected to participate in this study. Kumasi is the capital city of the Ashanti Region of Ghana and the second largest city in Ghana and has a population of about 1.2 million with about half of this being women (CIA, 2010).

3.2 STUDY POPULATION AND SAMPLING TECHNIQUES

3.2.1 Sampling Areas

The study recruited previously unscreened women from three major hospitals in the Kumasi Metropolis, namely Kumasi South Regional Hospital, Tafo-Government Hospital and Suntreso Hospital. Figure 7 is a map of Kumasi showing the relative strategic locations of the three study centres and the various sub-metros around which health services directorate in the metropolis is organised.

Located in the Asokwa sub-metro, the Kumasi South Regional Hospital (KSRH), which is one of three Cervicare clinics in the Region is located between three towns (Atonsu,

Agogo and Chirapatre) in the Ashanti Region of Ghana and provides services to 56 communities with approximately 650,372 residents (Table 3.1) (KMHD, 2013). The Kumasi South Regional Hospital serves the people of Asokwa, Ahensan, Atonsu, Esreso, Gyenyase and Kaase (KMHD, 2013)

The Tafo Government Hospital is located at Tafo in the Manhyia North sub-Metropolis. The Hospital serves as one of the major health facilities for people living in the northern part of the Kumasi metropolis and beyond. The Kumasi Metro Health Directorate reports that in 2013, the hospital had a catchment size of approximately 343,431 residents in the Manhyia North area (Table 3.1) (KMHD, 2013).

The Suntreso Government Hospital, located at the North-Suntreso area in Kumasi was established in 1963 as an urban health centre to provide primary healthcare to residents of the Bantama sub-metropolitan area and its environs. The Suntreso Government Hospital is located at North Suntreso and serves North and South Suntreso, Patasi Estate, Kwadaso, Adoato, Asuoyeboa, Breman and Suame with approximately 519,439 residents (Table 3.1) (KMHD, 2013).. The Ghana health services in 1996, upgraded the existing polyclinic to a District hospital to enable it handle more challenging health issues in the Bantama Sub-metro and its environs (KMHD, 2013).

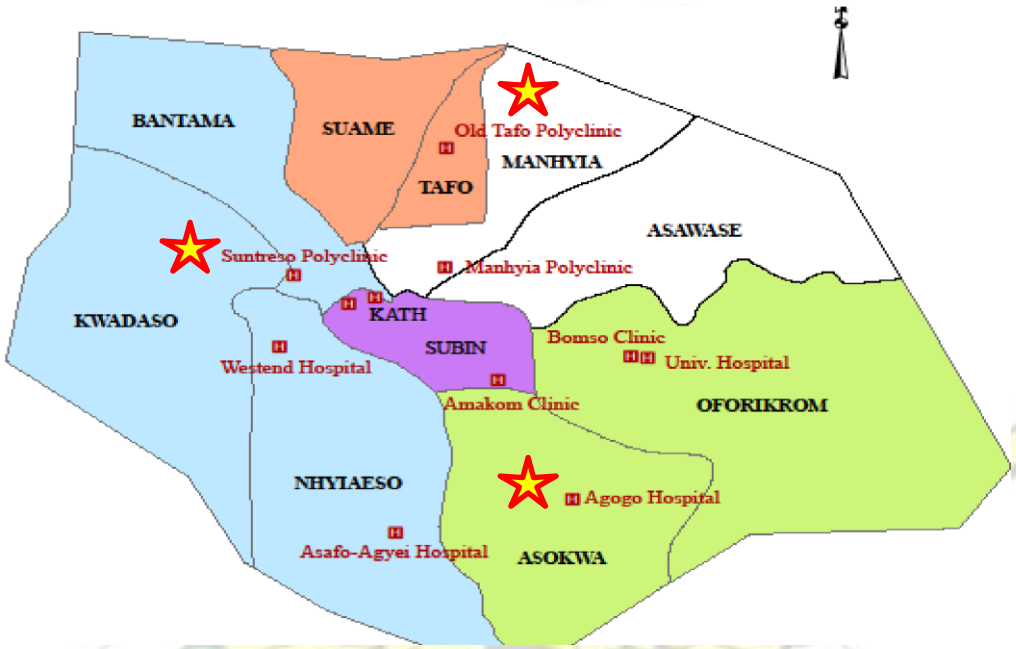

Figure 3.1: Health Facilities in Kumasi. Source: Pehr and Akuamoa-Boateng (2010).

Table 3.1: Table showing populationsizes and sample size distribution (N= 600).

Cervicare centre	Av. Annual OPD attendance*	Proportionate sample (n)
Kumasi South Hospital	159307	185
Tafo Hospital	144824	168
Suntreso Hospital	212177	247

Note: * the average annual attendance is calculated from figures for 2010, 2011 and 2012 calendar years. ***Source***: KMHD, 2013.

3.2.2 Sampling Technique

The Ghana Health Service has three established cervical screening centres in the Ashanti Region of Ghana. Women participating in this study were sampled from the out-patient departments (OPDs) of all of these three so-called Cervicare centres situated in Kumasi using a special systematic probability-proportional-to-size sampling (PPS) technique. In PPS sampling, the selection probability for each element is set to be proportional to its size measure. In this context, the probability of recruiting a woman into the study will be proportional to the catchment size of the Cervicare centre she attends. The PPS approach can improve accuracy for a given sample size by concentrating sampling on large hospitals under the assumption that they have the greatest impact on population estimates.

The three selected health facilities together serve 65.8% of the total Kumasi population and 70.5% of the total population of women of fertility age (WIFA). In addition, public OPD announcements were also carried out in the selected hospitals throughout the designated study period. The study was explained to eligible women and those who consented by appending their signature (or thumbprint, in the presence of a witness) were recruited. The respective average OPD attendance and calculated sample quotas from all three facilities are as shown in Table 3.1 above.

3.2.3 Sample Calculation and Sample Size

The approach used here to calculate sample size is based on the concept of precision of a reported sample statistics: that is the ability to estimate sample statistics that do not differ from the true population parameter by more than the required level of precision.

Here we have set the desired level of precision at ±5%. Assuming a prevalence of HPV of 37.1% in the general population of women (Schmitt *et al.*, 2013b), and using a

population of 1,529,151 for Kumasi (KMHD, 2013), a maximum sample size of 500 was estimated to ensure that the study will have enough precision to provide reliable statistics close enough to the true population parameters (Figure 8). This figure has been rounded up to the nearest hundred to cater for missing and incomplete data entries and other unforeseen circumstances.

3.2.1 Inclusion Criteria

Women attending the three clinics were contacted to take part in the study. . All women who consented by appending their signature (or thumbprint, in the presence of a witness) were recruited.

3.2.2 Exclusion Criteria

Women who were pregnant had undergone hysterectomy or conisation, or could not undergo an interview or speculum examinations were excluded from the study. Women who reported to have ever had a Pap smear before were also essentially excluded from the study.

SAMPLE SIZE CALCULATOR FOR PREVALENCE STUDIES

* USING RANDOM (NOT CLUSTER) SAMPLING

Level of Confidence = 95%
Expected *P* = 0.37
Population Size (*N*) = 1,529,151

* Suggested precision (d) is 0.05.

Sample Size Table

Precision (*d*)	Sample Size (*n*)		Suggestion for FPC application	Assumption (Normality)
	No FPC	With FPC		
± 0.01	8955		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.02	2239		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.03	995		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.04	560		◄ n/N≤0.05. FPC is NOT needed.	OK
► ± 0.05	359		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.06	249		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.07	183		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.08	140		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.09	111		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.10	90		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.11	75		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.12	63		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.13	53		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.14	46		◄ n/N≤0.05. FPC is NOT needed.	OK
± 0.15	40		◄ n/N≤0.05. FPC is NOT needed.	OK

Figure 3.2: Sample size calculation. Source: Naing *et. al.,* (2006).

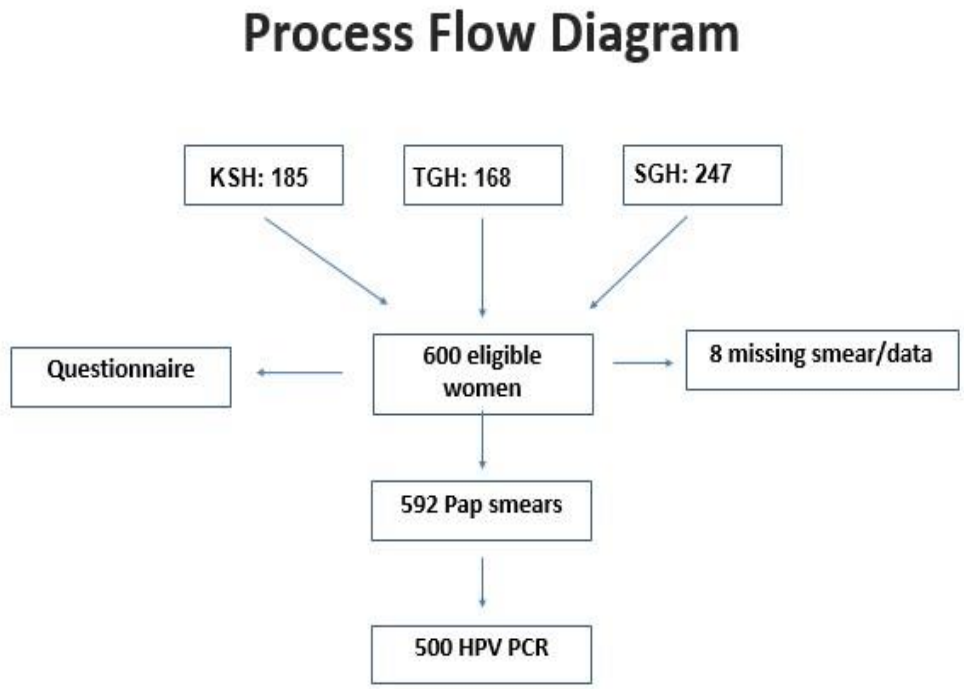

Figure 3.3: Process Flow Chart for study methods

3.3 DATA COLLECTION

3.3.1 Data Collection Tools: Pretesting and Administration

An in-depth, semi-structured, interviewer-administered questionnaire 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 the questionnaire for optimum achievement of study aims and benefits. The pre-tested questionnaire, which was in English, was transcribed to "Twi" (the most spoken and well understood dialect in the Region) by teaching assistants at the Department of Akan, Kwame Nkrumah University of Science and Technology and rendered by the investigator in the presence of a study nurse as required. The "Twi" version of the instrument is shown in Appendix 1b. The investigator ensured that respondents understood the questions first, before allowing them to give their responses. Information was sought on demographics, socioeconomic characteristics, tobacco use, reproductive and menstrual factors such as parity, sexual habits of the woman and her husband, and lifetime use of contraceptive methods.

3.3.2 Reliability and Validity of Questionnaire

Reliability in the context of data collection instruments is the extent to which a data collection instrument will yield consistent findings under constant conditions at all times. The test re-test approach of checking reliability (Mitchell, 1996) was adopted to ensure that the questionnaire used in this study was reliable. The questionnaire used in this study was administered on two separate occasions to 15 women at sample

collection centres during the pilot testing stage under very similar conditions in order to

check the consistency of the responses obtained. Questions that elicited inconsistent responses were either replaced or omitted altogether.

Validity is the extent to which a data collection method accurately measures what they are intended to measure (Bell and Waters, 2014). The face validity of the questionnaire was examined by interviewing people face-to-face, after they had completed the questionnaire to find out whether the responses they had given in the questionnaire agreed with their real opinions during the interviews. This was done at the pilot testing stage of the questionnaire to ensure its accuracy in obtaining the right kind of data. Furthermore, by soliciting the views of experts and project supervisors the study ensured that questions introduced in the questionnaire were essential for the research objective number 5.

3.3.3 Precautions

To ensure that optimum specimens were collected for analysis, standard procedure for collecting cervical samples was followed (Chan *et al.*, 2002). Sampling was performed at least 5 days after the cessation of menstruation and at least two days after vaginal sexual intercourse. Patients who had to return at a later date for sampling owing to menstruation or prior engagement in coitus were advised not to use vaginal douche, pessary or any type of lubricant 24 hours prior to their next appointment.

In addition to patient-oriented precautionary measures, all study nurses were trained by a gynaecologist and orally examined during a trial period and before the commencement of actual study sampling. Apart from this, nurses were routinely reminded of the objectives of the study and study protocols were further explained to them as and when necessary by the PhD student who was always on-hand on all sampling days.

Furthermore, for adequate sampling in postmenopausal and multiparous women, in whom the transformation zone is often anatomically higher and not visualized, the kit used included an endocervical brush as recommended. All slides that were suspected to have fungal overgrowth that could affect proper interpretation were replaced with new ones.

3.4 SPECMEN COLLECTION AND LABORATORY ANALYSIS/TESTS.

3.4.1 Smear Preparation, Fixation and Transportation

Papanicolau (Pap) smear test was performed for all the women. Cervical samples were obtained from the cervix with Pap Pak® cytology kit (Medical Packaging Corporation, Camarillo, CA, USA) according to the manufacturer's instructions 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 wet-fixed by spraying 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 transported to the Cytology Laboratory of the School of Allied Health Sciences, University of Ghana,

Korle-Bu, Accra 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.

3.4.2 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, all fixed smears arriving in the lab were left in 95% ethanol overnight to remove the carbowax protective layer, passed through a second alcohol wash to ensure complete removal of carbowax and primed by hydrating in running tap water for 4 minutes.

Slides were arranged in racks and lowered into Mayer's haematoxylin nuclear staining in batches before blueing in alkaline running water for 3 minutes each. Another priming step was performed by washing slides in 95% ethanol for 2 minutes. Afterwards, slides were stained in Orange G stain for 2 minutes and rinsed with 95% ethanol followed by staining with Eosin Azure.

Finally, slides were dehydrated in two changes of absolute ethanol 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 then allowed to dry on bench before screening for intra-epithelial lesions.

Cervical smears 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 an experienced pathologist for final interpretation, reporting and quality control.

3.4.3 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).

3.4.4 DNA Sample Collection

Exfoliated cervical cells left on the spatula and cytobrush after preparation of the Pap smears were recovered from the spatula by washing 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.

3.4.5 HPV-DNA Extraction, Detection and Genotyping

DNA was extracted with a commercial kit (Qiagen Ltd, Maryland, USA) according to the manufacturer's instructions as shown in Appendix 7. Briefly, cervical cells were lysed by incubation at 56^{o}C with 20µl QIAGEN Proteinase K in a proprietary buffer AL (300 µl). Next, the exposed DNA was primed by addition of 400 µl ethanol (96–100%) and bound to a special matrix in 2ml spin columns. The sample was then successively washed with buffer AW1 and AW2 by centrifuging at 8000 rpm for 1 min and 14,000 rpm for 3 min respectively. Finally bound, purified DNA was eluted into 50µl Buffer AE and stored at -70^{o}C in duplicate until further processing.

The DNA lysate prepared was quality controlled using human beta-globulin polymerase chain reaction (PCR) as described by de Roda Husman et al., (1995).

Briefly, for a PCR volume of 25µl, 1µl – 4µl of the DNA lysate and 25pmol of each human beta-globulin consensus primers PCO3+ and PCO4+ (Integrated DNA Technologies, Inc, USA) were used.

HPV-DNA detection and identification of the genotypes was carried out by nested multiplex PCR (NMPCR) (Sotlar et al., 2004). A single consensus forward primer (GPE6-3F) and two consensus back primers (GP-E7-5B and GP-E7-6B) were used for the 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 extracts was used as template for the amplification reactions.

This was carried out using a thermal cycler (BIOER GenePro thermocycler, BIOER Co., USA). The cycling parameters for the first round PCR with E63F/E75B/E76B consensus primers were as follows: 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 2). The other parameters that were used in the first round PCR mix were maintained. However, the cycling parameters were as follows: 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).

3.4.6 Analysis of Amplification Products

The amplification products were analysed 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 for the electrophoresis. Hundred base pair DNA molecular weight marker (Sigma, MO, USA) was run alongside the PCR products. The gel was prepared and electrophoresed in 1X TAE buffer using a mini gel system at 100 volts for one hour and photographed over UV illuminator (Sotlar *et al.*, 2004).

3.5 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.

The goals of the statistical analysis were 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 2 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 graphically and with simple contingency tables.

Exploratory analysis was carried out to obtain descriptive statistics. Categorical variables were compared using Chi square test whilst 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.

3.6 ETHICS APPROVAL

The study was approved by the Committee on Human Research, Publication and Ethics (CHRPE), Kwame Nkrumah University of Science and Technology, School of Medical Sciences (KNUST-SMS) and Komfo Anokye Teaching Hospital (KATH), Kumasi, the Ghana Health Service (GHS) through the Kumasi Metropolitan Health Directorate and the School of Allied Health Sciences (SAHS), University of Ghana, Korle-Bu, Accra. Study participants were adequately informed of the purpose, nature, procedures, risks and hazards of the study. Strict emphasis was placed on anonymity, confidentiality and the freedom to decline to participate at any time without penalty. All participants signed informed consent forms according to the recommendations of the local ethics review committees that gave approval.

Informed consent: Samples were collected after obtaining informed consent from the participants. Participation was strictly voluntary and any participant was free at any time to withdraw from the study without compromising their right to medical care.

Confidentiality: All data was handled anonymously and other information was expected to remain reasonably confidential.

Safety procedures: Samples were collected strictly by trained medical personnel who had had more than a years' experience in taking cervical smears.

Chapter 4 RESULTS

4.1 Demographic Profile of Study Population

This study investigated the association of selected demographic and behavioural characteristics with the detection of 18 low-risk and high-risk genital human papillomavirus (HPV) in women recruited in Kumasi for cervical screening. Cervical samples were obtained from 595 women from age 19 to 93 years. The mean age of the participants was 42.3 years (SD=11.6 years) and median age 42 years. Age and all other continuous variables studied were found to follow a Gaussian distribution according to D'AgostinoPearson normality test.

Figure 8 shows the demographic characteristics of the study population including the age profile. 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 (82.8%), Mole-Dagbani (10.3%), Ewe (3.7%) and Ga-Adangbe (2.6%). Illiteracy was reported by 11.8% of the women in study. At the time of the study, 2.9% of women were still in various stages of formal school education. However, the greater fraction of the population had completed middle school/junior high school (40.7%) while 15.9% had accessed tertiary level education. More than half of women were married (54.6%). Single women accounted for 12.1% of the study participants. With regards to employment/working status, 12.5% of women were unemployed while the rest were mostly traders (42.2%) or in some other self-employment (14.3%), public sector employees (11.9%) and private sector employees (5.6%).

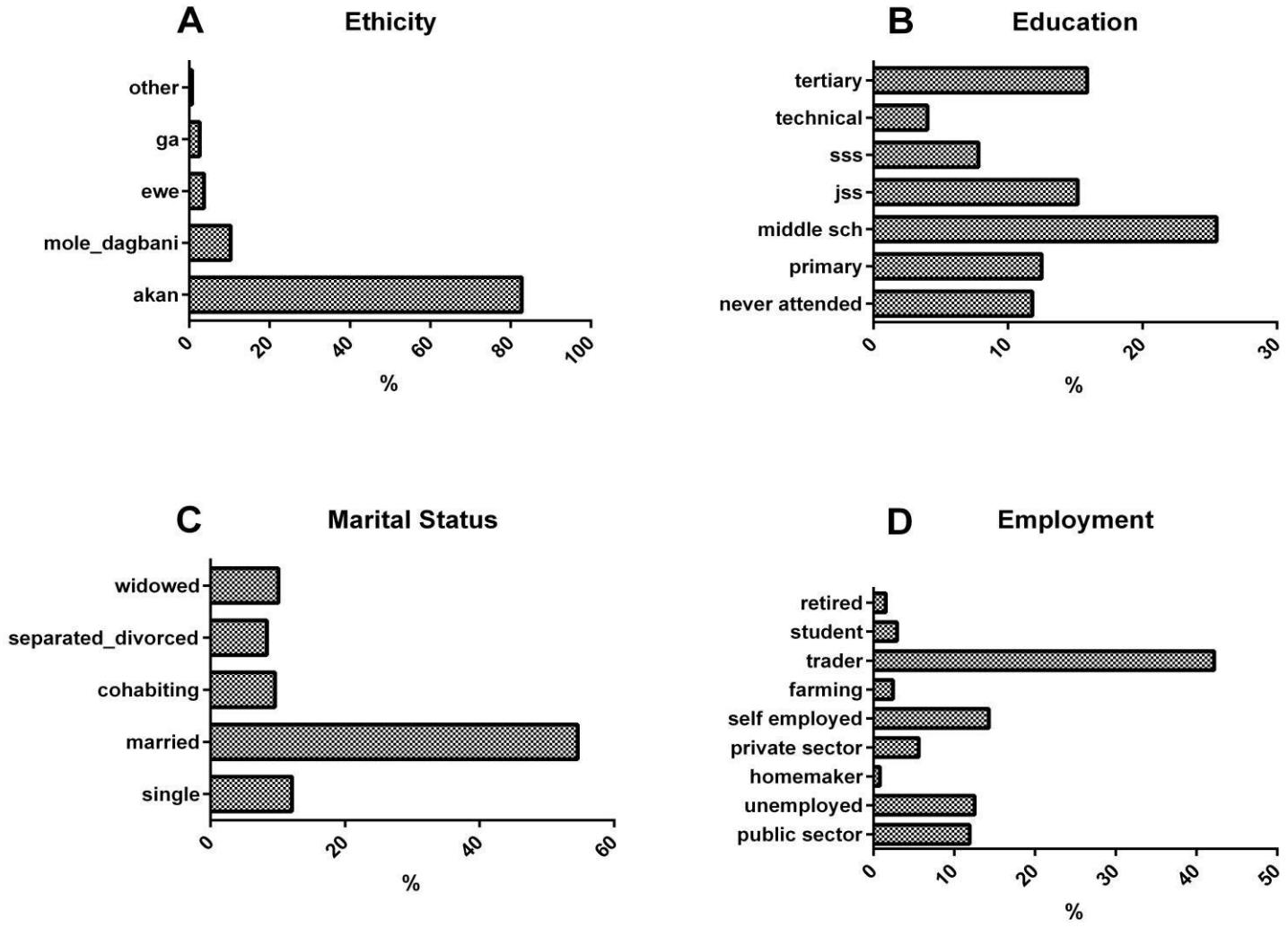

Figure 4.1: Demographic Characteristics of Study Population

Only 5.9% of women had never been pregnant. The average number of lifetime pregnancies was 4.5 (SD=2.8) whereas a few women (2.7%) reported from 10 to a maximum of 14 lifetime pregnancies. Almost half of the women (45.3%) reported five pregnancies or more. The mean number of all abortions reported per woman was 1.74 (SD=2.8) and the overall abortion prevalence rate was 73.8%. Additionally, 52% of all women reported having induced at least one abortion while 37.2% reported ever suffering at least one spontaneous abortion in their lifetime. These rates were calculated as mutually exclusive events since the occurrence of an induced abortion did not necessarily preclude a spontaneous abortion in any woman. Thus estimates were obtained by computing the total number of women reporting at least one of each specific type of abortion and dividing by total number of women.

The study documented the ages at onset and cessation of menses, age at first pregnancy and sexual experience. The average reported age of menarche was 15.25 years ranging from 11 to 24 years (SD = 1.93). The average reported age of menopause was 48.36 years ranging from 34 to 60 years (SD = 5.58). In all, 33.1% of respondents had their first sexual encounter before 18 years. Furthermore, approximately one in three women (29.0%) reported becoming pregnant for the first time by age 20 years. However, the average reported age for first conception was 21.30 years ranging from 12 to 36 years (SD = 4.42).

Approximately 80.3% of respondents had more than one sexual partner in their entire lifetime. Overall, there was a reported average of 3 lifetime sexual partners per woman ranging from 1 to 15 lifetime partners (median = 3.0). Additionally, 16.5% of married women suspected that that their husbands had had extramarital sexual relationships; 27.1% intimated that their husband's had been married before and 27.2% of married women were found in polygamous relationships. Forty eight per cent of the women had a history of

contraception. Out of this fraction, 57.1% reported to have used oral contraceptive methods and condom use was reported by 25.4% of the study women. However, tobacco intake was reported by a mere 1.6% of the population while alcohol consumption was reported by 36.4% of the women.

4.2 Prevalence of Cervical Cytology Abnormalities

Out of the 592 women for whom a Pap smear was available for evaluation, 555 (93.6%) were negative for intraepithelial lesion or malignancy. This category also includes smears with metaplastic changes (13, 2.2%) and smears without transformation zone components but no dyskaryosis seen either (NTZC_NDC: 110 women, 18.6%) (Table 4.1). It is worthy of note that although the NTZC_NDC smears were negative, there is the likelihood that some may be false negatives since sampling of the transformation zone cannot be guaranteed. In addition, 8 (1.4%) showed atypical squamous cells of undetermined significance (ASCUS), 9 (1.4%) low-grade squamous intraepithelial lesions, and 2 (0.3%) high-grade squamous intraepithelial lesions. Additionally, 3 women had squamous cell carcinoma. None of the women was found to have atypical glandular cells or adenocarcinoma. Other conditions including infections were detected among the population. Among all women screened, 43 had non-specific cervicitis (7.3%), 12 had candidiasis alone (2%), 12 had bacterial vaginosis alone (2%) and 1 had *Trichomonas vaginalis* (0.2%) infection.

Table 4.1: Prevalence of cervical abnormalities among women cytologically screened in Kumasi, Ghana, May 2012 to November 2014

Cervical cytology status		
	Frequency (N)	Percentage (%)
NILM*	555	93.6
ASCUS	8	1.4
LSIL	9	1.5
HSIL	2	.3
SCC	3	.5
Unsatisfactory	15	2.5
Total	592	100.0

NILM: Negative for intraepithelial lesions or malignancy. *This category also includes smears with metaplastic changes and smears without transformation zone components but no dyskaryosis seen either, ASCUS: Atypical squamous cells of unknown significance, LSIL: Low-grade squamous intraepithelial lesions, HSIL: high-grade squamous intraepithelial lesions, SCC: squamous cell carcinoma.

4.3 Prevalence of HPV Genotypes among Unscreened Women

In the present study cervical swabs were obtained from 500 women with no history of Pap smear screening and analysed for 18 HPV types. Table 4.2a shows the Prevalence of human papillomavirus (HPV) types detected in cervical specimens from these women. Also shown is the prevalence of low-risk HPV (types 6/11, 42, 43, 44 and 66) (Table 4.2b) and highrisk HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) (Table 4.2c) in the specified population.

The prevalence of HPV oncogenic DNA of any type among the study population was 37.2% (95% CI: 33.4 - 41.6). The prevalence of LR HPV was 14.2% (95% CI: 11.3 – 17.6). (38.2% of HPV positive cases) while that of HR HPV was 31.4% (95% CI: 27.4 – 35.7). (84.4% of HPV positive cases).

Table 4.2a: Prevalence of human papillomavirus (HPV) in 500 women 18–93 years old cytologically screened in Kumasi, Ghana, May 2012 to November 2014.

Parameter	**n**	**%[a]**	**%[b]**
HPV negative	314	62.8	
HPV positive	186	37.2	
Low-risk HPV only[α]	29	5.8	15.6
High-risk HPV only[β]	115	23.0	61.8
Both high and low-risk	42	8.4	22.6
Multiple HPV infections	85	17.0	45.7

[a] Percentages calculated using total number of cases (N= 500) as common denominator. [b] Percentages calculated using number of positive cases (N= 186) 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. Tables 4.2b. and 4.2c show the relative frequencies of HPV types detected in the study. The commonest HR types were, HPV-52

Table
(11.6%; 58 out of 500 women), HPV-56 (7%; 35 out of 500 women), HPV-35 (5%; 25 out of 500 women), HPV-18 (4.8%; 24 out of 500 women), HPV-66 (3.8%; 19 out of 500 women), and HPV-58 (3.2%; 16 out of 500 women). HPV 42, a low-risk type, was also common (7.8%; 39 out of 500 women). The frequency of HPV 16 (the commonest genotype associated with cervical cancer worldwide) detection was 1.4% (7 out of 500 women).

Table 4.2b: Prevalence of high-risk human papillomavirus (HPV) Genotypes in 500 women 18 years and above cytologically screened in Kumasi, Ghana, May 2012 to November 2014

HPV type	**n***	**%[a]**	**%[b]**	**%[c]**
HPV-52	58	11.6	31.2	38.4
HPV-56	35	7.0	18.8	23.2
HPV-35	25	5.0	13.4	16.6
HPV-18	24	4.8	12.9	15.9
HPV-58	16	3.2	8.6	10.6
HPV-68	16	3.2	8.6	10.6
HPV-51	12	2.4	6.5	7.9
HPV-39	11	2.2	5.9	7.3
HPV-45	9	1.8	4.8	6.0
HPV-16	7	1.4	3.8	4.6
HPV-59	3	0.6	1.6	2.0
HPV-33	3	0.6	1.6	2.0
HPV-31	2	0.4	1.1	1.3

* Because of multiple infections, women may be counted more than once. [a] Percentages calculated using total number of cases (N= 500) as common denominator. [b] Percentages calculated using number of positive cases (N= 186) as common denominator [c] Percentages calculated using number of HR cases (N= 157) as common denominator.

4.2c: Prevalence of low-risk human papillomavirus (HPV) Genotypes in 500 women 18 years and above cytologically screened in Kumasi, Ghana, May 2012 to November 2014

HPV type	n*	%[a]	%[b]	%[c]
HPV-42	39	7.8	21.0	54.9
HPV-43	30	6.0	16.1	42.3
HPV-66	19	3.8	10.2	26.8
HPV-6/11	9	1.8	4.8	12.7
HPV-44	3	0.6	1.6	4.2

* Because of multiple infections, women may be counted more than once. [a] Percentages calculated using total number of cases (N= 500) as common denominator. [b] Percentages calculated using number of positive cases (N= 186) as common denominator [c] Percentages calculated using number of LR cases (N= 71) as common denominator.

Study population demographics and human papillomavirus (HPV) genotype prevalence estimates are presented in Table 4.3 for all infected women. Row percentages are computed to show within-group HPV prevalence estimates. The estimated age-related prevalence rates ranged from 32% among women aged 55-64 years to 47.6% among women from the age 65 and above category. Chi-square test was performed to test the significance of the difference in the detection frequency of the genotypes in different age groups. However, no significant difference was detected across the different age categories for low-risk (χ^2=8.70, degrees of freedom (df)= 5, P=0.12), high-risk (χ^2=2.39, degrees of freedom (df)= 5, P=0.79) and any type-HPV (χ^2=2.84, degrees of freedom (df)= 5, P=0.72) detection. Agespecific HPV DNA prevalence estimates for low-risk and high-risk infection and any typeHPV are shown in Table 4.3. Prevalence rates are calculated as row percentages in SPSS to show within-group HPV estimates. Women who were cohabiting with their partners had a lower overall HPV prevalence (32%) compared to formally married women (35%), single women (47%) and currently divorced/bereaved women (37%). But the

Table

differences were not significant (χ^2=3.60, df= 3, P=0.31). However, the highest detection rate for high-risk

infections (100%) and the lowest detection rate for low-risk infections was among the cohabiting women. There was no significant difference in HPV prevalence across the different ethnic classes (χ^2=0.97, df=3, P=0.92). However, the Mole-Dagbani group recorded the highest prevalence rates for all-type HPV (40.5%) and high-risk HPV (88.2%). Additionally, there were no statistically significant differences in HPV detection across the various strata for number of conceived pregnancies, parity, number of abortions, age at first pregnancy and age at coitache (Tables 4.3 and 4.4).

Table

4.3: Study population demographics and human papillomavirus (HPV) prevalence among women screened in Kumasi, Ghana, May 2012 to November 2014.

Characteristic	No. (%*)	Prevalence of HPV, %#		
		Low-risk HPV	High-risk HPV	Any HPV
Age				
<25	20 (4.1)	33.3	77.8	45.0
25-34	120 (24.3)	31.9	87.2	39.2
35-44	154 (31.2)	32.1	86.8	34.4
45-54	128 (26.0)	36.7	81.6	38.3
55-64	50 (10.1)	68.8	75.0	32.0
≥65	21 (4.3)	50.0	90.0	47.6
	N=493	*8.70; 0.12*		
Education				
never attended	51 (11.0)	41.7	87.5	47.1
primary	60 (13.0)	25.9	81.5	45.0
middle / JHS	202 (43.7)	33.8	84.6	32.2
SHS	41 (8.9)	23.1	92.3	31.7
technical_vocational	21 (4.5)	44.4	66.7	42.9
tertiary	87 (18.8)	50.0	84.4	36.8
=5; P-value)	***N=462***	*5.55; 0.35*	*3.06; 0.69*	*6.69*
Marital status				
single	64 (13.4)	43.3	83.3	46.9
divorced/widowed	81 (17.0)	43.3	73.3	37.0
married	281 (59.0)	35.4	84.8	35.2
cohabiting	50 (10.5)	18.8	100.0	32.0
a)	***N=476***	*3.43;0.33*	*5.65;0.13*	
Ethnicity				
Akan	383 (83.4)	38.6	82.1	36.6
Mole-dagbani	42 (9.2)	35.3	88.2	40.5
Ewe	17 (3.7)	0	100.0	29.4
Ga	13 (2.8)	20.0	80.0	38.5
alue)	***N=459***		*1.89; 0.76*	
Parity				
gravidae				
0	34 (7.4)	25.0	91.7	35.3
1 to 2	86 (18.7)	48.4	74.2	36.0
3 to 4	135 (29.3)	28.3	87.0	34.1
≥5	206 (44.7)	38.0	84.8	38.3
	N=461			

[#] Row percentages are computed to show within-group HPV prevalence estimates.

[*]Percentage fraction of respondents**.**

Table 4.3 continued

Characteristic	No. (%[*])	Prevalence of HPV, %[#]		
		Low-risk HPV	High-risk HPV	Any HPV
para				
0	65 (14.1)	25.0	87.5	36.9
1 to 2	150 (32.5)	40.0	81.8	36.7
3 to 4	156 (33.8)	37.5	83.9	35.9
≥5	90 (19.5)	38.2	82.4	37.8
	N=461	*1.72; 0.63*		
abortion				
0	125 (27.2)	48.7	79.5	31.2
1	118 (25.7)	33.3	85.7	35.6
≥2	216 (47.1)	32.6	84.9	39.8
[illegible]; df=2, P-value)	***N=459***	*3.27; 0.20*	*0.72*	*2.58; 0.2*
Age at first pregnancy (years)				
≤17	66 (16.9)	20.0	95.0	30.3
18-21	162 (41.4)	36.2	81.2	42.6
22-25	94 (24.0)	50.0	80.6	38.3
>25	69 (17.6)	31.6	78.9	27.5
[illegible]	***N=391***	*5.36; 0.15*	*2.53; 0.47*	

[#] Row percentages are computed to show within-group HPV prevalence estimates.

[*]Percentage fraction of respondents**.**

Human papillomavirus (HPV) detection rates among women are summarized in Table 4.4. according to categories of sexual history variables of interest. At the 5% level of significance, HPV DNA detection was significantly associated with ***lifetime sex partners*** ***(χ^2=3.92, df=1,P=0.048), extramarital activity of woman's partner (χ^2=7.34, df=2, P=0.026),*** but not with ***woman's age at coitache, polygamy marital history of woman's partner*** and ***main partner's age.*** As expected, the concurrent visual inspection with acetic

Table
acid (VIA) report was highly correlated with the detection of any type HPV (χ^2=13.1, df=1, P=0.000) and less strongly with low-risk and high-risk HPV positivity.

4.4: Sexual history and human papillomavirus (HPV) prevalence among women screened in Kumasi, Ghana, May 2012 to November 2014.

Characteristic	No. (%)	Prevalence of HPV, %[#]		
		Low-risk HPV	High-risk HPV	Any HPV
Age at Coitache				
≤15	39 (10.4)	41.7	91.7	30.8
16-18	152 (40.5)	31.7	88.9	41.4
19-21	121 (32.3)	35.6	82.2	37.2
≥22	63 (16.8)	35.3	82.4	27.0
	N=375	*0.51; 0.92*		
lifetime sex partners[a]				
Single	81 (20.9)	43.5	73.9	28.4
multiple	307 (79.1)	33.9	85.5	40.4
(χ^2, df=1, P-value)	***N=388***	*0.78; 0.38*	*1.91; 0.17*	*3.92; 0.04*
Main partner ever married				
No	139 (54.3)	42.9	73.8	30.2
Yes	117 (45.7)	26.7	95.6	38.5
	N=265	*2.52; 0.11*	*8.08; 0.*	
Husband with unmarried (extramarital) partners [a]				
No	162 (63.0)	38.0	84.0	30.9
Yes	47 (18.3)	43.8	75.0	34.0
Not sure	48 (18.7)	28.0	92.0	52.1
df=2, P-va	***N=257***	*1.19; 0.55*	*2.[illegible]*	*7.34; 0*
Wife of polygamous **relatio**	**ıship**			
No	117 (43.5)	35.7	88.1	35.9
Yes	152 (56.5)	36.5	80.8	34.2
alue)	***N=269***		*0.93; 0.34*	
Main partner's age ≤				
34	33 (12.5)	18.2	90.9	33.3
35-44	83 (31.3)	42.9	75.0	33.7
45-54	87 (32.8)	21.9	93.8	36.8
55-64	47 (17.7)	47.1	82.4	36.2
≥65	15 (5.7)	20.0	80.0	33.3
	N=265			

VIA[a]				
Normal	465 (93.0)	38.0	83.4	35.1
Abnormal	35 (7.0)	39.1	91.3	65.7
(χ^2, df=1, P-value)	***N=500***	*0.01; 0.92*	*0.95: 0.33*	*13.1: 0.00*

[#] 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 (*lifetime sex partners, P=0.048; Husband with unmarried (extramarital) partners, P=0.026; VIA, P=0.000*).

4.3.1 Age-Trend of HPV infections

Figure 4.2 shows HPV prevalence estimates for all women with normal cytology. Women with abnormal cervical findings were excluded to facilitate comparison with other studies. For this same reason, the age categories 55-64 and >65 years have been merged as one group, >55 years. HPV prevalence was highest in women younger than 25 years and prevalence decreased in the 35–44 year-group. A second but smaller peak in HPV infections occurred in the 45–54 year-group followed by another decline.

Table

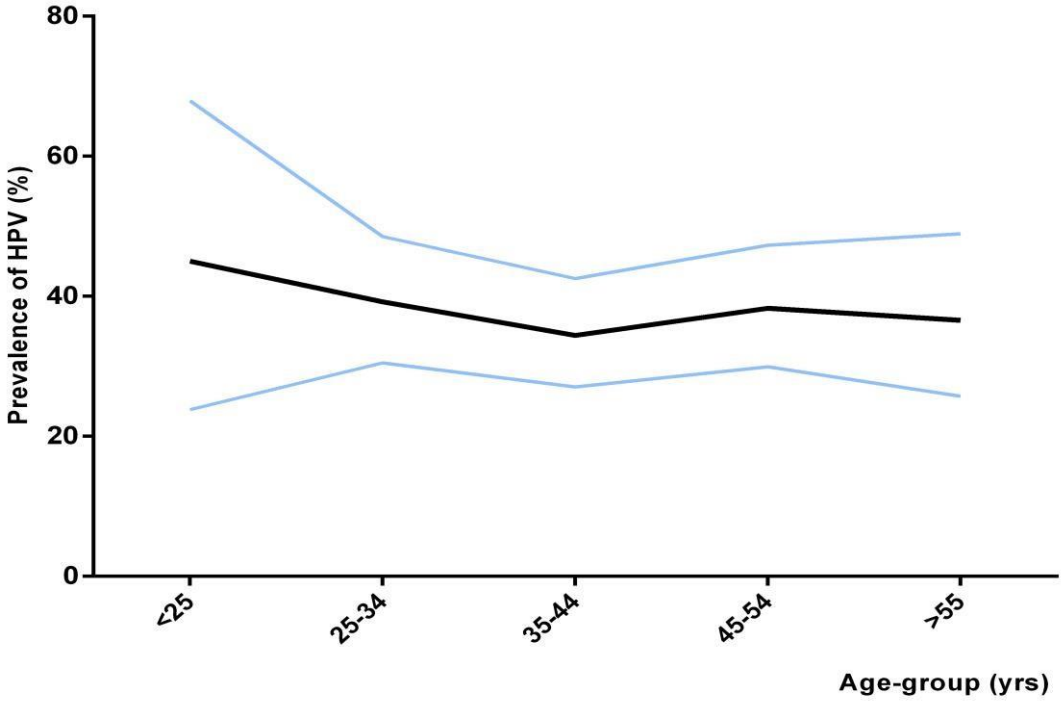

Figure 4.2: Age-specific HPV prevalence among women with normal cytology (coloured lines represent upper and lower 95% CIs).

4.4 Prevalence and Distribution of HPV genotypes in Cervical Disease

Table 4.5 is an overview of HPV type distribution in Kumasi stratified according to concurrent cytology outcome and number of detected HPV genotypes. The total number of women with an LR HPV genotype infection (irrespective of the presence of other HPV types) was calculated as a percentage of the total number of cases (n= 500) and stratified under two categories: single and multiple infections. The same was done for HR HPV. The results are presented based on the presence or absence of cervical cytological dysplasia.

The majority of women (473 women: 94.6%) had normal cervical findings. Only twenty seven women (5.4%) had a concurrent abnormal Pap smear result. Among women who had abnormal cervical results, the prevalence of HPV infection was 62.9% (17 women) compared to those who had a normal cytology result (35.7%, 169 women) (χ^2=7.6, df=1, P=0.006). As expected, women who had abnormal Pap smear findings had a preponderance of HR HPV infection (χ^2=10.3, df=1, P=0.001). The prevalence of HR HPV infection was 59.3% among women with abnormal simultaneous pap result compared to 29.8% among women with normal Pap smear findings. The distribution of LR HPV infections according to cytology outcome however, was not significant (χ^2=1.51, df=1, P=0.219). Again, women with atypical pap smears had a greater tendency to harbour multiple HR HPV infections compared to women with normal pap smears (χ^2=7.12, df=1, P=0.008).

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Table 4.5 HPV type distribution in Kumasi stratified according to concurrent cytology outcome and number of detected HPV genotypes HPV Prevalence and cytological outcomes for 500 women screened in Kumasi, Ghana, May 2012 to November 2014.

HPV type	Pap test								
	Normal			Abnormal			Total		
	Single	Multiple	Total (%)	Single	Multiple	Total (%)	Single	Multiple	Total (%)
			n= 473 (94.6)			*n= 27 (5.4)*			*n= 500*
HPV negative			304 (64.3)			10 (37.0)			314 (62.8)
HPV positive	97	72	169 (35.7)	4	13	17 (62.9)	101	85	186 (37.2)
HR HPV positive [α]	72	69	141 (29.8)	3	13	16 (59.3)	75	82	157 (31.4)
LR HPV positive [β]	25	40	65 (13.7)	1	5	6 (22.2)	26	45	71 (14.2)

[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.

Table 4.6: Prevalence of LR HPV types by cytological diagnosis for 500 women screened in Kumasi, Ghana, May 2012 to November 2014.

	Pap test								
	Normal			Abnormal			Total		
HPV type	**Single**	**Multiple**	**Total (%)**	**Single**	**Multiple**	**Total (%)**	**Single**	**Multiple**	**Total (%)**
			n= 473 (94.6)			***n= 27 (5.4)***			
Low-risk infections									
42									
	16	18	34 (7.2)	0	5	5 (18.5)	16	23	39 (7.8)
43	9	19	28 (5.9)	1	1	2 (7.4)	10	20	30 (6.0)
66	5	12	17 (3.6)	0	2	2 (7.4)	5	14	19 (3.8)
44	0	3	3 (0.6)	0	0	0	0	3	3 (1.8)
6/11	0	8	8 (1.7)	0	1	1 (3.7)	0	9	9 (0.6)
sub-total	**30**	**60**	**90 (19.0)**	**1**	**9**	**10 (37.0)**	**31**	**69**	**100 (20.0)**

Table 4.7: Prevalence of HR HPV types by cytological diagnosis for 500 women screened in Kumasi, Ghana, May 2012 to November 2014

	Pap test		
	Normal	Abnormal	Total

	Single	Multiple	Total (%)	Single	Multiple	Total (%)	Single	Multiple	Total (%)
			n= 473 (94.6)			***n= 27 (5.4)***			
High-risk infections									
52									
	18	31	49 (10.4)	0	9	9 (33.3)	18	40	58 (38.4)
35	11	11	22 (4.7)	1	2	3 (11.1)	12	13	25 (16.6)
18	11	8	19 (4.0)	1	4	5 (18.5)	12	12	24 (15.9)
58	2	12	14 (3.0)	0	2	2 (7.4)	2	14	16 (10.6)
56	10	22	32 (6.8)	0	3	3 (11.1)	10	25	35 (23.2)
39	1	9	10 (2.1)	0	1	1 (3.7)	1	10	11 (7.3)
51	4	8	12 (2.5)	0	0	0 (0.0)	4	8	12 (7.9)
59	0	2	2 (0.4)	1	0	1 (3.7)	1	2	3 (2.0)
45	1	5	6 (1.3)	0	3	3 (11.1)	1	2	9 (6.0)
16	2	2	4 (0.8)	0	3	3 (11.1)	2	5	7 (4.6)
68	6	8	14 (3.0)	0	2	2 (7.4)	6	10	16 (10.6)
33	0	2	2	0	1	1 (3.7)	0	1	3 (2.0)
sub-total	**66**	**120**	**186**	**3**	**30**	**33**	**69**	**150**	**219 (43.8)**

Results

4.5 Prevalence of low risk, high risk and any type HPV in Cervical Cytology

The present study investigated the relationship between detection of HPV genotypes and severity of cytological lesions in previously unscreened women across Kumasi from 2010 to 2012. Table 4.8 presents the distribution of all types of HPV, low risk and high risk HPVs in concurrent cervical cytology outcomes for these women. Prevalence of HPV infection ranged from 37.9% among women with normal Pap findings to 100% among women with HSIL and SCC. The cross-tabulation procedure in SPSS allows an answer to be provided to the question of association between the various cytology outcomes and the prevalence of low risk, high risk and any type HPV among women in Kumasi. The concurrent Pap smear report was highly correlated with the detection of any type HPV and less strongly with low-risk and high-risk HPV types (Table 4.8).

Table 4.8: Distribution of low risk, high risk and any type HPV in concurrent cervical cytology outcomes for 500 women screened in Kumasi, Ghana, 2012-2014.

Characteristic	No. (%#)	Prevalence of HPV, %*		
		Any HPV[a]	High-risk HPV	Low-risk HPV
NILM	472 (94.4)	36.4	83.1	39.0
ASCUS	7 (1.4)	71.4	100.0	20.0
LSIL	8 (1.6)	75.0	100.0	16.7
HSIL	2 (0.4)	100.0	100.0	50.0
SCC	2 (0.4)	100.0	100.0	50.0
(χ^2, df=4, P-value)		*19.5; 0.00*	*2.80; 0.59*	*3.66; 0.45*

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 (Pearson x^2 test).

The prevalence odds ratio is a measure of the percentage of persons in a population who have some outcome of interest at a particular point in time in one group of subjects/individuals (with

a specific characteristics/attribute) relative to another group (without the characteristics/attributes). The prevalence odds ratios (pORs) of an abnormal pap result given the detection of specific HPV groups, (using a normal diagnosis as the reference) were as follows. For high-risk HPV types, the pOR of an abnormal pap was 3.2 (95% CI = 0.4 – 24.9); low-risk HPV types was 0.8 (95% CI = 0.3 – 2.5) and all type HPV was 2.9 (95% CI = 1.3 – 6.6). The prevalence of HPV (any type) varies significantly across categories (P =0.002, Pearson x^2 test).

Table 4.9: Multiple logistic regression on prevalence odds ratios (pORs) of cytology outcome following the detection of specific human papillomavirus (HPV) infection.

		Prevalence odds		
Pap smear result	**No.**	**LR-HPV pOR (95% CI)**	**HR-HPV pOR (95% CI)**	**Any HPV pOR (95% CI)**
Normal	473	1	1	1
Abnormal	27	0.8 (0.3 – 2.5)	3.2 (0.4 – 24.9)	3.0 (1.3 – 6.6)*
NILM	356	1	1	1
ASCUS *(β=1.38, df=1, P=0.10)*	7	0.4 (0.1 – 3.6)	1.2 (0.1 – 10.4)	4.0 (0.8 – 20.9)
LSIL *(β=1.57, df=1, P=0.06)*	8	0.4 (0.1 – 2.7)	1.2 (0.2 – 11.8)	4.8 (1.0 – 24.1)

* Predictor variable has significant overall effect on outcome variable (β=1.08, df=1, P=0.00)
β: Coefficient of regression; df: degrees of freedom

The prevalence odds ratios (pORs) of cytology diagnoses given the detection of specific HPV groups, (using a normal outcome as the reference), were also computed. The pOR for atypical squamous cells of unknown significance (ASCUS) and low-grade squamous intraepithelial lesions (LSILs) given an existing HR HPV infection was not significantly different from the odds for either having a high-grade squamous intraepithelial lesion (HSIL) or squamous cell carcinoma (SCC) (Table 4.9). The same was observed for LR HPV infection. However, for all type HPV infection, the pORs of ASCUS was 4.0 (95% CI = 0.8 – 20.9), LSIL was 4.8 (95% CI = 1.0 – 24.1), HSIL was 3.2 (95% CI = 0.3 – 35.6), and SCC was 4.8 (95% CI = 0.5 – 46.5). In addition, the pOR for an abnormal Pap result was 3.0 (95% CI = 1.3 – 6.6) (P=0.008)(Table 4.9).

Table 4.10 shows the exclusive distribution of HPV genotypes in the 500 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 (25% of all ASCUS cases), 52 (25% of all ASCUS cases) and 68 (25% of all ASCUS cases). Among women with LSIL most prevalent were HPV-52 (66.5% of all LSIL cases), HPV-18 (22.2% of all LSIL cases) and HPV 45 (22.2% of all LSIL cases). HPV16 (50% of all HSIL cases), 52 (50% of all HSIL cases) and 42 (50% of all HSIL cases) were top three in that order for high grade intraepithelial/severe dysplasia lesions. HPV-16 (66.6% of all SCC cases) was more commonly detected in cases with suspected squamous cell carcinoma than any other genotype.

Table 4.10: Distribution of HPV genotypes in 500 women screened in Kumasi, Ghana, 20122014 according to concurrent cytological diagnosis.

HPV genotype	ASCUS	LSIL	HSIL	SCC	Total	95% CI
	N= 8 (%)	*N= 9 (%)*	*N= 2 (%)*	*N= 3 (%)*	*N= 27 (%)*	
High-risk infections						
52	2 (25)	6 (67)	1 (50)		9 (33)	17.2-53.9
35	1 (13)	1 (11)			2 (7)	1.3-25.8
18	2 (25)	2 (22)		1 (33)	5 (19)	7.0-38.8
58	1 (13)			1 (33)	2 (7)	1.3-25.8
56	1 (13)	1 (11)		1 (33)	3 (11)	2.9-30.3
39				1 (33)	1 (4)	0.2-20.9
51						
59						
45	1 (13)	2 (22)			3 (11)	2.9-30.3
16			1 (50)	2 (66)	3 (11)	2.9-30.3
68	2 (25)				2 (7)	1.3-25.8
33		1 (11)			1 (4)	0.2-20.9
Low-risk infections						
42	1 (13)	1 (11)	1 (50)	1 (33)	4 (15)	4.9-34.6
43				1 (33)	1 (4)	0.2-20.9
66		1 (11)		1 (33)	2 (7)	1.3-25.8
44						
6/11	1 (13)				1 (4)	0.2-20.9

Column percentages are displayed to show within-group type-specific HPV prevalence according to categories of concurrent cytological diagnosis.

4.6 Multiple human papillomavirus (HPV) infections, clustering tendency and cluster frequencies

Of the women positive for HPV, 101 out of 186 (54.3%) were infected with a single HPV type, and 85 (45.7%) were infected with more than one HPV type. The distribution of multiple HPV clusters among the 85 multiple HPV cases was as follows: 47 out of 85 instances of multiple infection (55.2%) were with 2 types, 26 (30.6%) were with 3 types, 9 (10.6%) were with 4 types and 3 (3.5%) were with 5 types. Combinations of HPV types found in women with multiple HPV infections are given in Table 4.11.

The association between specific HPV genotype detected in cervical smears and multiple infection status are shown in Table 4.12 Out of the 18 HPV types screened in this study, HPV-52 was the commonest genotype involved in multiple infections but HPV-6/11, HPV-33, HPV-44, HPV-39, HPV-58 and HPV-45 had the higher risk estimate to cluster with other HPV types. HPV-16 and 18 however, were not likely to be associated with multiple infection status.

Table 4.11: Combinations of human papillomavirus (HPV) types in 85 women with multiple infections

Double		Triple	Four	Five
HPV cluster	**frequency**	**HPV cluster**	**HPV cluster**	**HPV cluster**
6/11, 52	2	16, 42, 52 *	16, 39, 56, 58 *	16, 18, 42,43, 66 *
16, 31		16, 6/11, 42	35, 42, 39, 51	18, 45, 56, 42, 39
18, 42		18, 35, 56	42, 45, 52, 6/11 *	42, 44, 39, 66, 51
18, 43	2	18, 42, 56	45,35,43,68	
18, 52 *		18, 43, 52	52, 56, 35, 43	
18, 68 *		18, 45, 52*	52, 56, 58, 68 *	
35, 42 *	2	18, 52, 68	52, 58, 6/11, 68	
35, 52 *	6	18, 56, 68	52, 6/11, 39, 51	
39, 42		33, 39, 66	56, 43, 44, 68	
39, 52	2	33, 45, 52*		
39, 66		35, 52, 56		
42, 43		39, 56, 58		
42, 44		42, 52, 56 *		
42, 45		42, 52, 58		
42, 52	2	42, 52, 59		
43, 51	2	42, 52, 66		
43, 52		43, 51, 66		
43, 56		43, 56, 66		
43, 59		43, 56, 66		
43, 66		43, 56, 66		
45, 52		45, 33, 58		
52, 56	5	52, 58, 42		
52, 58		52, 58, 66		
52, 66 *	2	6/11, 42, 52		
52, 68		6/11, 43, 51		
56, 58	4	6/11, 43, 51		
58, 68	2			

*Associated with abnormal cervical finding.

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

Table 4.12: The association between specific HPV genotype detected and multiple infection status

HPV type	Multiple[a] (%) (N = 85)	Single[b] (%) (N = 101)	RR[c] (%)	95 % CI
HPV-6/11	9	0	2.35	1.98-2.79
HPV-33	3	0	2.23	1.90-2.62
HPV-44	3	0	2.23	1.90-2.62
HPV-39	10	1	2.12	1.65-2.73
HPV-58	14	2	2.09	1.62-2.71
HPV-45	8	1	2.04	1.54-2.72
HPV-52	40	18	1.96	1.47-2.63
HPV-56	25	10	1.8	1.35-2.40
HPV-66	14	5	1.73	1.26-2.39
HPV-43	20	10	1.6	1.17-2.19
HPV-16	5	2	1.6	0.97-2.63
HPV-51	8	4	1.51	0.98-2.32
HPV-59	2	1	1.47	0.65-3.32
HPV-68	10	6	1.42	0.93-2.15
HPV-42	23	16	1.34	1.01-1.93
HPV-35	13	12	1.16	0.77-1.76
HPV-18	12	12	1.11	0.72-1.71
HPV-31	1	1	1.1	0.27-4.42

[a] Percentage of total multiple-infections

[b] Percentage of total single-infections

[c] RR: relative risk is a ratio of event probabilities. The relative risk measures the odds of the association between being positive for each type HPV and the state of multiple-infection

4.7 Correlates of abnormal cytology and their implications for disease prevention

Tables 4.13 and 4.14 show the odds ratios (ORs) for Pap smear abnormality and corresponding 95% confidence intervals (CIs) according to socio-demographic and reproductive characteristics among women.

Table 4.13: Odds ratios (ORs) for cervical dysplasia and corresponding 95% confidence intervals (CIs) according to socio-demographic characteristics of women

			Pap smear abnormality		
	N	*%#*	*β (p-value)*	*OR*	*95% CI*
Age a*					
≤40	268	3.0		1.0	
>40	306	8.5	1.11 (0.01)	3.0	1.3-6.8
(χ^2=7.79, df=1, P=0.01)					
Education					
never attended	67	6.0		1.0	
primary	73	4.1	-0.39 (0.61)	0.7	0.1-3.1
middle / JHS	238	5.9	-0.01 (0.97)	1.0	0.3-3.1
SHS	46	6.5	0.09 (0.91)	1.1	0.2-5.2
technical_vocational	24	0.0	-18.4 (0.99)		
tertiary	93	8.6	0.39 (0.54)	1.4	0.4-5.1
Marital status					
single	71	5.6		1.0	
divorced/widowed	106	6.6	0.17 (0.79)	1.2	0.3-4.2
married	319	6.3	0.11 (0.84)	1.1	0.4-3.4
cohabiting	58	3.4	-0.51 (0.56)	0.6	0.1-3.3
Ethnicity					
Akan	445	6.3		1.0	
Mole-Dagbani	56	3.6	-0.59 (0.43)	0.6	0.1-2.3
Ewe	19	5.3	-0.19 (0.85)	0.8	0.1-6.4
Ga	14	0.0	-18.5 (0.99)		

[a] Predictor variable has significant overall effect on outcome variable.
* Prevalence of abnormal cytology varies significantly across categories (P=0.008; Pearson x^2 test).
\# Row percentages computed to show within-group prevalence for cervical dysplasia.

As anticipated, age had overall significant predictive effect on Pap smear abnormality (P=0.008). In addition, older women (>40 years old) had significantly increased odds (OR=3.0; 95% CI: 1.3-6.8) for an abnormal cervical cytology compared to younger women. Educational status did not significantly alter a woman's odds for an abnormal smear when comparing less and non-educated women to highly educated women. Married women had an OR of 1.1 (95% CI: 0.4-3.4), being divorced or widowed did not seem to be associated with an increased percentage of abnormal Pap smear results compared to being currently single.

History of having a greater number of pregnancies was associated with a higher but not significant prevalence odds ratio for Pap smear abnormality: OR=2.1 (95% CI: 0.3 – 18.3) for 1-2 lifetime pregnancies and 3.0 (95% CI: 0.4 – 23.5) for 5 or more lifetime pregnancies compared to nulligravidae women. Neither increasing delay in the onset of menstruation nor young age at first pregnancy was significantly associated with Pap smear positivity (Table 4.14). The prevalence of abnormal cervical cytology was directly affected by a woman's number of reported abortions (x^2 test P=0.015) and abortion status had significant effects for predicting cervical dysplasia (P=0.034). Compared to abortion-naïve women, women who had had even one aborted pregnancy had higher odds for abnormal cervical cytology 0R=4.2 (95% CI: 0.9 – 20.0). Having a reproductive history of at least 2 abortions increased the odds further to 6.5 (95% CI: 1.5 – 28.2). The age at first pregnancy did not significantly affect a woman's cytology status.

The mode (single vs. multiple infection) and identity (vaccine preventable or not) of HPV infection were significant predictors of cervical dysplasia (P=0.013 and P=0.042 respectively) (Table 4.14). As expected, having concurrent multiple HPV infection was associated with a significantly higher prevalence odds ratio for Pap smear abnormality, OR=4.4 (95% CI: 1.4-

14.0) compared to single infection. Additionally, simultaneous infection with both 9-valent vaccine preventable HPV genotypes and other HPV types excluded from the vaccine had higher prevalence odds ratio for Pap smear abnormality OR=4.1 (95% CI: 1.3-13.9) (P=0.042), compared to having only 9-valent vaccine types, OR=1.6 (95% CI: 0.3-7.3).

Table 4.15 extends the odds ratios (ORs) for Pap smear abnormality and corresponding 95% confidence intervals (CIs) to sexual history characteristics and concurrent VIA reports of women. There was no significant relationship between woman's age at sexual debut, lifetime number of sexual partners, women whose partners were previously married and women in polygamous husbands and abnormal cervical cytology. However, for married women, the age of a woman's sexual partner had a significant relationship with her Pap smear result (P=0.026). As expected, the concurrent VIA report was a significant predictor of cervical dysplasia in general (P=0.000). Having an abnormal VIA was associated with an OR of 10.5 (95% CI: 4.8-23.0) compared to normal VIA (P=0.000).

Table 4.14: Odds ratios for cervical dysplasia (95%CIs) and reproductive characteristics

	Pap smear abnormality				
	N	***%[1]***	***β (p-value)***	***OR***	***95% CI***
Gravidae *(χ^2=4.11, df=3, P=0.25)*					
0	35	2.9		1.0	
1 to 2	102	5.9	0.75 (0.49)	2.1	0.3-18.3
3 to 4	160	3.8	0.28 (0.79)	1.3	0.2-11.4
≥5	243	8.2	1.12 (0.28)	3.0	0.4-23.5
Para *(χ^2=3.43, df=3, P=0.33)*					
0	70	1.4		1.0	
1 to 2	177	7.3	1.69 (0.11)	5.5	0.7-42.6
3 to 4	186	7.0	1.65 (0.12)	5.2	0.7-40.4
≥5	106	5.7	1.42 (0.19)	4.1	0.5-35.2
Abortion [a]* *(χ^2=8.39, df=2, P=0.01)*					
0	141	1.4		1.0	
1	141	5.7	1.43 (0.07)	4.2	0.9-20.0
≥2	246	8.6	1.88 (0.01)	6.5	1.5-28.2
Age at first pregnancy (years) *(χ^2=3.01, df=3, P=0.39)*					
≤17	79	10.1		1.0	
18-21	191	6.8	-0.43 (0.36)	0.6	0.3-1.6
22-25	115	5.2	-0.72 (0.20)	0.5	0.2-1.5
>25	79	3.8	-1.05 (0.13)	0.4	0.1-1.4
Mode of HPV infection [a]* *(χ^2=7.14, df=1, P=0.01)*					
single	101	4.0		1.0	
multiple	85	15.3	1.48 (0.13)	4.4	1.4-14.0
Type of HPV infection *(χ^2=1.54, df=2, P=0.46)*					
LR	29	3.4		1.0	
HR	115	9.6	1.09 (0.31)	2.9	0.4-24.0
both	42	11.9	1.33 (0.24)	3.8	0.4-34.2
Vaccine Preventable infection [a]**(χ^2=6.54, df=2, P=0.04)*					
No	85	4.7		1.0	

[1] Row percentages computed to show within-group prevalence for cervical dysplasia [a] Predictor variable has significant overall effect on outcome variable (*abortion, P=0.034; mode of infection, P=0.013; vaccine preventable infection, P=0.042*) *Prevalence of abnormal cytology varies significantly across categories (*abortion, P=0.015; mode of infection, P=0.015; vaccine preventable infection, P=0.038*).

Yes	42	7.1	0.44 (0.57)	1.6	0.3-7.3
both	59	16.9	1.42 (0.02)	4.1	1.3-13.9

s 4.15: Odds ratios (ORs) for cervical dysplasia (95% CI) according to sexual history of women.

	Pap smear abnormality				
	N	***%#***	***β (p-value)***	***OR***	***95% CI***
Age at Coitache					
≤15	50	12.0		1.0	
16-18	187	5.9	-0.78 (0.15)	0.5	0.2-1.3
19-21	144	4.2	-1.14 (0.05)	0.3	0.1-1.0
≥22	70	4.3	-1.11 (0.13)	0.3	0.1-1.4
lifetime sex partners					
Single	86	8.1		1.0	
multiple	350	6.3	0.28 (0.54)	1.3	0.5-3.2
Main partner ever married *34, df=1, P=0.25)*					
No	153	3.3		1.0	
Yes	130	6.2	0.66 (0.26)	1.9	0.6-6.1
Husband with extramarital partners *0.01, df=2*					
Not sure	66	4.5		1.0	
No	174	4.6	-0.07 (0.94)	0.9	0.2-5.8
Yes	47	4.3	0.01 (0.99)	1.0	0.3-3.9
Wife of polygamous relationship *(χ^2=0.89, df=1 P=0.35)*					
No	147	3.4		1	
Yes	159	5.7	0.53 (0.35)	1.7	0.6-5.2
Main partner's age* *(χ^2=11.07, df=4, P=0.03)*					
≤ 34	36	2.8		1.0	
35-44	99	0.0	-17.6 (0.99)	0.0	
45-54	106	7.5	1.05 (0.33)	2.8	0.3-23.6
55-64	54	11.1	1.47 (0.18)	4.3	0.5-37.9
≥65	19	5.3	0.67 (0.65)	1.9	0.1-32.9
VIA[a]* *(χ^2=49.8, df=1, P=0.00)*					
Normal	540	4.1		1.0	
Abnormal	42	31.0	2.36 (0.00)	10.5	4.8-23.0

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

*Associated with a significant p-value for Pearson chi square test (*main partner's age, P=0.026; VIA, P=0.000*).

Table 4.113

[a] Predictor variable has significant overall effect on outcome variable (*P=0.000*).

4.8 Correlates of genital HPV infection and their implications for disease prevention

Tables 4.16 and 4.17 show the odds ratios (ORs) for HPV positivity and corresponding 95% confidence intervals (CIs) according to socio-demographic and reproductive characteristics among women. At the 5% level of significance, age, educational status, marital status and ethnic grouping were not significant independent predictors of HPV positivity. Illiteracy was not associated with an excess odds of HPV-positivity compared to women who had completed various levels of education. There were no significant trends in HPV prevalence for different numbers of birth, pregnancies or abortions. Odds ratios for different number of births were very similar to those for number of pregnancies.

However, HPV positivity was significantly associated with sexual history variables of women. Multiple lifetime sex partners (P=0.048) and having a husband with extramarital sexual partners (P=0.029) were independent significant predictors of HPV infection among women. Having multiple lifetime sexual partners was associated with an OR of 1.7 (95% CI: 1.1-2.9) for an HPV infection compared to women with single lifetime partners (P=0.049).

As expected, the concurrent VIA report was a significant predictor of any type HPV detection in general (P=0.001). Having an abnormal VIA was associated with an OR of 3.6 (95% CI: 1.7-7.3) for an HPV infection compared to normal VIA (Table 4.18).

: Odds ratios (ORs) for HPV positivity and corresponding 95% confidence intervals (CIs) according to socio-demographic characteristics of women

		HPV positivity			
Characteristic	***N***	***% #***	***β (p-value)***	***OR***	***95% CI***

Age					
<25	21	45.0		1.0	
25-34	147	39.2	-0.24 (0.62)	0.3	0.05-1.36
35-44	185	34.4	-0.44 (0.36)	0.2	0.04-1.10
45-54	146	38.3	-0.28 (0.57)	0.2	0.03-0.93
55-64	62	32.0	-0.55 (0.31)	0.1	0.02-0.59
≥65	24	47.6	0.11 (0.87)	0.3	0.03-2.24
Education					
never attended	70	47.1		1.0	
primary	74	45.0	-0.08 (0.83)	0.9	0.4-1.9
middle / JHS	241	32.2	-0.63 (0.06)	0.5	0.3-0.9
SHS	46	31.7	-0.62 (0.10)	0.5	0.2-1.2
technical_vocational	24	42.9	-0.65 (0.14)	0.8	0.3-2.4
tertiary	94	36.8	-0.17 (0.75)	0.7	0.3-1.3
Marital status					
single	64	46.9		1.0	
divorced/widowed	81	37.0	-0.41 (0.23)	0.7	0.3-1.3
married	281	35.2	-0.48 (0.08)	0.6	0.4-1.1
cohabiting	50	32.0	-0.63 (0.11)	0.5	0.3-1.2
Ethnicity					
Akan	383	36.6		1.0	
Mole-Dagbani	42	40.5	0.17 (0.62)	1.2	0.6-2.3
Ewe	17	29.4	-0.32 (0.55)	0.7	0.3-2.1
Ga	13	38.5	0.08 (0.88)	1.1	0.3-3.4

Row percentages computed to show within-group prevalence for HPV positivity

Table 4.17

: Odds ratios (ORs) for HPV positivity and corresponding 95% confidence intervals (CIs) according to reproductive characteristics among women

Characteristic	HPV positivity				
	N	***%*** [#]	***β (p-value)***	***OR***	***95% CI***
Parity					
Gravidae					
0	34	35.3		1.0	
1 to 2	86	36.0	0.03 (0.94)	1.0	0.5-2.4
3 to 4	135	34.1	-0.05 (0.89)	0.9	0.4-2.1
≥5	206	38.3	0.13 (0.73)	1.1	0.5-2.4
Para 0	65	36.9		1.0	
1 to 2	150	36.7	-0.01 (0.97)	1.0	0.5-1.8
3 to 4	156	35.9	-0.04 (0.89)	1.0	0.5-1.7
≥5	90	37.8	0.04 (0.91)	1.0	0.5-2.7
Abortion					
0	125	31.2		1.0	
1	118	35.6	0.20 (0.47)	1.2	0.7-2.0
≥2	216	39.8	0.39 (0.11)	1.5	1.1-2.3
Age at first pregnancy (years)					
≤17	66	30.3		1.0	
18-21	162	42.6	0.53 (0.09)	1.7	1.2-3.1
22-25	94	38.3	0.36 (0.30)	1.4	0.7-2.8
>25	69	27.5	-0.14 (0.72)	0.9	0.4-1.8

Row percentages computed to show within-group prevalence for HPV positivity

Table 4.18

: Odds ratios (ORs) for HPV positivity (95% CI) according to sexual history of women.

	HPV positivity				
	N	***%#***	***β (p-value)***	***OR***	***95% CI***
Age at Coitache					
≤15	39	30.8		1.0	
16-18	152	41.4	0.47 (0.23)	1.6	0.8-3.4
19-21	121	37.2	0.29 (0.47)	1.3	0.6-2.9
≥22	63	27.0	-0.18 (0.68)	0.8	0.3-2.0
lifetime sex partners[a*]					
Single	81	28.4		1.0	
multiple	307	40.4	0.54 (0.04)	1.7	1.1-2.9
Main partner ever married					
No	139	38.5		1.0	
Yes	117	30.2	0.37 (0.17)	1.4	0.8-2.4
Husband with unmarried (extramarit	**ıl)** **ɔartners**	**[a*]**			
		52.1		1.0	
Not sure	48				
No	162	30.9	-0.75 (0.07)	0.4	0.2-0.8
Yes	47	34.0	-0.89 (0.00)	0.5	0.2-0.9
Main partner's age ≤					
34	33	33.3		1.0	
35-44	83	33.7	0.02 (0.98)	1.0	0.4-2.4
45-54	87	36.8	0.15 (0.72)	1.2	0.5-2.7
55-64	47	36.2	0.13 (0.79)	1.1	0.4-2.9
≥65	15	33.3	0.00 (1.00)	1.0	0.3-3.7
VIA[a*]					
Normal	465	35.1		1.0	
Abnormal	35	65.7	1.27 (0.00)	3.6	1.7-7.3

Row percentages computed to show within-group prevalence for HPV positivity

*Associated with a significant p-value for Pearson chi square test (*lifetime sex partners, P=0.048; Husband with extramarital sexual partners, P=0.026; VIA, P=0.000*).). a Predictor

variable has significant overall effect on outcome variable (*lifetime sex partners, P=0.049; Husband with unmarried (extramarital) partners, P=0.029; VIA, P=0.021*).

Chapter 5
DISCUSSION

5.1 Prevalence of Cervical Cancer and Pre-cancerous Lesions

This study represents the most up to date report on the prevalence of cervical abnormalities among women screened in Kumasi, Ghana. The overall prevalence of cervical lesions of 3.7% (22/592 women, Table 4.1) reported here in women presenting for Pap smear screening is within the range of what has been previously reported in the Region and from a coordinated study in two separate communities (Agogo (12.6%) and Nkawie (3.5%)) with similar objective (Handlogten *et al.*, 2014). However, in that study, volunteer women were recruited to participate by local hospital employees and physicians and previously screened women were not excluded. Heterogeneous sampling methods could account for the discordance in abnormality rates observed between the two locations and with the current study. It is anticipated that Ghana, and for that matter Kumasi, has an unusually high prevalence estimate for cervical cancer and pre-cursor lesions in the world since there is no organized cervical cancer prevention programme in place. Ghana does not have a national cervical cancer screening program currently and Pap smears are routinely ordered for differential diagnosis in cases of abnormal vaginal bleeding rather than as part of a routine gynaecologic examination or screening program (Adanu, 2002)

The reason why population sampling is crucial to the reported prevalence estimate is that cervical dysplasia and cancer is caused by persistent HPV infection but approximately 90%

Table 4.20

of infections clear spontaneously within 24 months (Schiffman *et al.*, 2007). So even though most women become infected with the virus following a sexual experience, the infection resolves spontaneously. Consequently, only a small proportion of women develop

cervical epithelial cell abnormalities over time, usually not less than 10 years. As a result of this relatively small proportion of women, a major challenge of population–based cervical screening studies is to provide sufficiently representative samples with adequate power to detect all grades of cervical dysplasia. Consequently, where some categories are not detected in a study, questions arise over suitability of sampling technique, size and test sensitivity. Although in this study, all levels of extracellular cell abnormalities according to the Bethesda nomenclature were detected, local studies that failed to detect some categories are not uncommon (Chen *et al.*, 2005; Handlogten *et al.*, 2014).

We have here reported prevalence estimates of 1.4% for atypical squamous cells of undetermined significance (ASCUS), 1.4% for low-grade squamous intraepithelial lesions, 0.3% for high-grade squamous intraepithelial lesions and 0.5% for squamous cell carcinoma (Table 4.1). A study by Hangloten *et al.* (Handlogten *et al.*, 2014), did not detect women with ASCUS or ICC among 255 women screened in Nkawie. Only HSIL (2.0%) and LSIL (1.6%) were detected even though previously screened women were not excluded. In another study in the capital, 843 Pap smears were evaluated to estimate the prevalence of cervical cancer and dysplasia among women. Seven hundred and sixty women (90.1%) had normal smears whereas 8.7% were classified as ASCUS, 0.6 % as LSIL and 0.6% were diagnosed as HSIL and no cases of cervical cancer were detected (Chen *et al.*, 2005). These reports may be explained partly by small size of sample, systematic bias in sample selection, and inexperience of clinical staff especially when the majority of the Pap smears are prepared by staff under training, resulting in a high number of unsatisfactory slides (Chen *et al.*, 2005).

Additionally, when estimating the prevalence of cervical cancer and precursor lesions, it is equally important that the sampling method used yields a sample with a normal age distribution. It is an established fact that cervical dysplasia occurs in elderly women secondary to HPV-induced cellular changes that accumulate over time (Fonn *et al.*, 2015). As a result of the time period required to induce severe dysplastic changes, young women are usually less likely than the elderly to have poorer Pap smear results. The high rate of early-stage dysplastic lesions (ASCUS/LSIL) and the low prevalence of cancerous smears may be attributed to the fact that the age distribution reported in that study is skewed to the left. In the current study the ages of women screened follow a Gaussian distribution and the estimate of 0.5% for cervical cancer is consequently higher (Table 4.1).

The diagnostic frequency of cervical cancer among women in this study and other local cross-sectional studies (Chen *et al.*, 2005; Handlogten *et al.*, 2014) suggest that cervical cancer may be less common in the population than previously anticipated. The only notable exception is a retrospective study conducted among women admitted into the gynaecologic unit of Korle-bu Teaching Hospital, Nkyekyer et al., (2000) documented a prevalence of 1.6% for cervical cancer exclusive of other precancerous lesions. Data from global estimates suggest that women from *developing countries* have a risk of 1.5% to develop cervical cancer before age 65 compared to their counterparts in *developed countries* (0.8%) (Parkin and Bray, 2006; WHO/ICO, 2010a).

Although the Pap Smear test has a very good specificity for malignancy and precursor lesions, the corresponding low sensitivity of the test (Saslow *et al.*, 2012) has been cited as a probable reason for the low diagnostic frequencies observed in screening studies (Chen *et al.*, 2005). Even in settings routinely screening women for cervical abnormalities, test

sensitivities ranging from as low as 42% to 73% is commonly reported (Sawaya *et al.*, 2001; ALTS, 2003; Castle *et al.*, 2010; Wright *et al.*, 2014). Thus, using the Pap test alone, a high proportion of cases may be misdiagnosed with serious clinical implications. Cervical cancer screening strategies that integrate HPV testing may improve disease detection and afford longer screening cycles (Saslow *et al.*, 2012). Comparatively, the HPV test has greater sensitivity but less specificity diagnosing women with CIN3+ (Arbyn *et al.*, 2006). To address this deficiency in progressive clinical settings, one of two protocols may be followed regarding cytology screening. The first practice has been to perform reflex HPV testing for all ambiguous cytology reports (ASCUS) and onward colposcopic evaluation in the event of a positive HPV screen. The second more expensive approach is employed in only a few resource-endowed laboratories and involves cytology and HPV co-testing approach (Wright *et al.*, 2014). Both approaches have been shown to improve the clinical performance of cytology in other settings.

To further improve the performance of the Pap smear, many laboratories use a diagnostic frequency ratio of ASC/SIL plus carcinoma (ASC/SIL+) as a quality management tool (Nascimento and Cibas, 2007). The ASC/SIL+ ratio provides some correction for patient population, because both ASC and SIL rates are expected to be higher among high-risk populations (Davey *et al.*, 2004) but there is no single recommended ASC/SIL+ ratio (Juskevicius *et al.*, 2001; Davey *et al.*, 2004). The ASC/SIL+ ratio found in the present study is lower than what has been documented in lower risk settings (Davey, 2005). In general, population samples with high proportion of high-risk women may have more

definitive SIL cases and show lower ASC/SIL+ ratios than laboratories with older or wellscreened women in whom the most abnormalities tend to be ASC (Davey, 2005).

A number of concordant estimates of the prevalence of epithelial cell abnormality (ECA) have been reported in other resource limited settings in sub-Saharan Africa: In a large community-based south African study (Fonn *et al.*, 2015), 2.42% of women had LSIL; 1.8% had HSIL and 0.47% had ICC. A cross-sectional report from Cameroon found a prevalence of 3.9% for cervical precancerous lesions (Marie *et al.*, 2013). In Burkina Faso, prevalence of precancerous lesion of the uterine cervix was 4.2% (Soudre *et al.*, 1992). Similar rates of ECA have been reported among Turkish women (Açikgöz and Ergör, 2010) and among Italian women (Meloni *et al.*, 2014).

The distribution of cervical cytology categories in this study is similar to the pattern of reporting rates from laboratory surveys in literature (Davey, 2005). The results of this study (Table 4.1) also support the assertion that the most common abnormality reported by cervical cytology correspond to atypical squamous cells (ASC) from the Bethesda classification (Davey, 2005). Additionally, the results are concordant with the notion that adenocarcinomas are much less common than SCC (Parkin and Bray, 2006). In fact, the proportion of adenocarcinoma detected in the present study was 0% (Table 4.1). In general, the fraction of adenocarcinoma cases is greater in settings with a low cervical cancer burden (Parkin *et al.*, 2002). Alternatively, the low fraction of adenocarcinomas could be a consequence of the screening methodology. The use of a cytobrush instead of an endocervical swab may diminish the probability of including glandular epithelial cells higher up the endocervical canal where by definition, cervical adenocarcinomas and related precursors occur (IARC, 2004).

Differences of prevalence of the precancerous lesions of the uterine cervix from one country to another is primarily due to the existence and consistency of screening programs and management options implemented in these countries (Parkin and Bray, 2006). In different regions of Nigeria, Pap smear screening have shown a lower prevalence of cervical ECA (7.6 − 13.2 %) (De Lemos *et al.*, 2012; Durowade *et al.*, 2012).However, difference in study methodology is always an important factor. Variation in ECA prevalence estimates may be the direct result of purposive sampling due to the inclusion of women at high risk of HIV-infection (Getinet *et al.*, 2015). HIV is known to be an important independent risk factor for development of precursor lesions and cervical cancer.

Apart from conventional cytology, visual examination using acetic acid or Lugol solution has also been used for cervical cancer screening in SSA (Albert *et al.*, 2012). In Rwanda, population-based cervical screening results based on VIA report prevalence of cervical cancer and precancer lesions was 1.7% and 5.9%, respectively. In Nigeria the prevalence of pre-cancer lesions was between 4.8-14% (Albert *et al.*, 2012). a pooled analysis of 19,579 women from Malawi, Madagascar, Nigeria, Uganda, Tanzania, and Zambia, the prevalence of invasive cancer was 1.7% (WHO, 2012).

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5.2 Prevalence and Pattern of HPV Genotypes among Women

HPV prevalence estimates give a measure of the fraction of individuals carrying a new, persistent, or recurring HPV infection in a population at a particular point in time. The proportion of high-risk HPV genotypes in the present study (84%) (Table 4.2a) was relatively high but consistent with studies involving women attending routine gynaecological screening. Said *et al.,* (2009), investigated the prevalence and distribution of HPV genotypes in women with normal and abnormal cervixes participating in a controlled study. Among women with normal cervixes, as high as 73% of all HPV-positive samples were HR-HPV types even though the women were mainly volunteers from the local community (Said *et al.*, 2009). In a cross-sectional study by Brandful *et al.* aimed to estimate HPV prevalence in pregnant women (18-41 years) in Ghana, overall HPV prevalence was 65% and of this number 72% were infected with high-risk HPV genotypes (Brandful *et al.*, 2014). Prevalence estimates can vary depending on the demographic and sexual behaviour patterns of the population under study. Therefore, differences in study population characteristics may account for the differences in result.

The variability in HPV detection rates demand that very robust and highly sensitive methods are desirable for establishing authoritative, reproducible estimates. Using a highlysensitive nested PCR assay that integrates degenerate E6/E7 consensus probes and typespecific probes, the prevalence of HPV infection found in this study was 37.2% (Table 4.2a). This figure is consistent with previous reports that more women harbor an HPV infection in Sub-Saharan Africa than in other parts of the world (Castellsague *et al.*, 2001; Xi *et al.*, 2003; Thomas *et al.*, 2004). Yar *et al.,* (2015) conducted a study on 107 women infected with HIV and 100 non-HIV-infected apparently healthy women as controls.

Overall HPV positivity for cases and controls was 86.9% and 56.0% respectively. The excess HPV positivity among control women may be explained by the expanded genotype portfolio used. In that study, 28 high- and low-risk HPV genotypes were characterized while the present study captured 18 HPV including all types of major interest.

HPV prevalence estimates from sub-Saharan Africa are generally high with a few isolated reports showing some variation. But oftentimes, the reported rates from different sources are difficult to compare directly owing to variation in sampling methods and assays used. As it is, both population surveys and purposive samples from clinical settings have been reported to use different molecular protocols operating at different sensitivities (Franceschi *et al.*, 2006). Also, estimates may be considerably discordant, owing to sample selection modalities (simple randomised sampling versus opportunistic series from hospital settings) and to the vast array of HPV DNA assays (Bosch *et al.*, 2006). Prevalence estimates based on the Hybrid Capture II assay range from 17% in rural Uganda (Serwadda *et al.*, 1999) to 25% among HIV-free women in urban Zimbabwe (Womack *et al.*, 2000b). PCR-based assays have estimated 40% HPV prevalence in rural Mozambique (Castellsague *et al.*, 2001), 31% in the capital of Zimbabwe (Gravitt *et al.*, 2002), 18% in Dakar and Pikene, Senegal (Xi *et al.*, 2003), and 44% in Nairobi, Kenya (De Vuyst *et al.*, 2003).

5.2.1 Dimensions to detected proportion of HPV 16 and 18 in HPV studies: General population versus cancer studies

Another interesting dimension of HPV studies is the frequency of detection of HPV 16 and HPV 18 (Table 4.2b). These two extremely high risk oncogenic genotypes are the two covered by the bivalent vaccine Cervarix. In this study, there was a similar combined rate of detection of HPV 16 and 18 oncogenes (6.2%), to the Brandful study in Ghana (6.6%

(Table 4.2b). Yar *et al*. (2015) also reported a low overall prevalence of HPV 16 and 18 similar to this study. This is a regular feature of HPV studies in the general population of previously unscreened cervixes and lies in sharp contrast to the scenario seen in global HPV studies involving histologically confirmed cancer tissue (Bosch and De Sanjosé, 2002; De Sanjosé *et al.*, 2007; Denny *et al.*, 2014). HPV16 and HPV18 infections are rarer in women with normal cytology compared to their significance in severe cervical lesions.

The higher prevalence of HPV 16 and HPV 18 in cancerous women compared with women who remain unscreened for cervical abnormalities reflect the tendency of HPV 16 and 18 to be more persistent in infection and induce more aggressive cell-level changes that predispose infected women to cervical dysplasia. It does not necessarily mean that existing bivalent/quadrivalent HPV vaccines may be ineffective in preventing a great fraction of HPV infections that would eventually result in cancer as some suggest. HPV16 and/or HPV18 are responsible for over 50% of the infections detected in HSIL, 70% of infections in ICC, and 81·5% of infections detected in adenocarcinomas (Munoz *et al.*, 2003b; Castellsague *et al.*, 2006). In a recent study by Awua and colleagues, HPV-specific DNA was detected in 89.8% of 230 tissue blocks from the archived histological repository at Korle-Bu in Accra (Awua *et al.*, 2016). The four commonly detected (overall prevalence) HPVs were HPV18, HPV59, HPV45 and HPV16. The same four HPV genotypes were the commonest infecting genotypes in both multiple and single infections but in different sequence. In a similar study of 50 tissue blocks accessed at the same hospital and using a similar assay, a prevalence of 98.0 % was reported for HPV (Attoh *et al.*, 2010). The commonest detected genotypes were HPV 18 (84%), HPV 16 (24%), HPV 45 (6%), and HPV 39 (4%).

A large multicenter study comprising women from sub-saharan Africa, who had cervical lesions suspected to be ICC has been published (Denny *et al.*, 2014). In that study, HPV positivity was 90.4% and the commonest detected genotypes were HPV16, 18, 45, 35, 33 and 52. The commonest HPV types reported by another study of cervical tumour samples in Cote d'Ivoire were HPV16, HPV18, HPV45, HPV35, and HPV31 (Adjorlolo-Johnson *et al.*, 2010). Similarly, a study in Benin reported HPV59 (24.6 %), HPV35 (22.5 %), HPV16

(17.6 %), and HPV18 (14.8 %) as the common HPV genotypes detected (Piras *et al.*, 2011). Also, in Burkina Faso, HPV52 (14.7 %), HPV35 (9.4 %), HPV58 (9.4 %), and HPV51 (8.6 %) were the common genotypes (Didelot-Rousseau *et al.*, 2006). Furthermore, several studies from other geographic regions in Africa and beyond confirm the point that although HPV16 and HPV18 are not the commonest genotypes in the general population of women, they are unequivocally the commonest genotype in cervical cancers globally.

However, that does not imply that HPV 16 and 18 are necessarily the two commonest HPVs in every country. The answer to that question is readily provided by studies such as the present one targeting the general female population. In the Benin and Burkina Faso studies mentioned above, the 2 commonest HPVs were not types 16 and 18 but HPV-59 and -35 and HPV-52 and -35 respectively. In Tanzania, the commonest genotypes were HPV 16 and HPV 58 (Mayaud *et al.*, 2003); in a Mozambique study, HPV35 was the most prevalent HPV genotype among women suspected to have HSIL or worse (Castellsague *et al.*, 2001) and HPV18 was not detected at all. Liaw et al., (1997) reported HPV52 and

HPV58 as the most prevalent type in parts of China. Assuming that the factors that contribute to the geographic disparities in HPV genotype proportions are related to

vaccination, a clearer understanding of what shapes the genotype distribution from one country to another might give us more clues about expected post-vaccination HPV genotype distributions in any population.

5.2.2 Age-Trend of HPV infections

A peculiar age-related trend of HPV infection was observed (Figure 5.1). HPV prevalence peaked early on (largely driven by high-risk genotypes) among women 25 years and younger and remained consistently high throughout the middle-aged group despite a decrease in the 35–44 year-group. A second peak in HPV infections occurred the 45–54 year-group followed by another decline in older women. The age trend of HPV found in Kumasi suggests that middle aged women retain a significantly elevated prevalence of HPV. Domfeh *et al.*, studied a cross-section of women selected from the outpatient clinic of the a tertiary Hospital in Accra, Ghana; and also found an elevated prevalence of HPV in older age groups. This trend may be a defining feature of populations where HPV transmission remains untamed among middle aged women as a consequence of polygamous arrangements and cervical cancer incidence is very high (Domfeh *et al.*, 2008). This age-related pattern of HPV infection has been similarly reported in populations with a generally high risk for cervical cancer, such as Ibadan in Nigeria (Thomas *et al.*, 2004) and Chennai in India (Franceschi *et al.*, 2003).

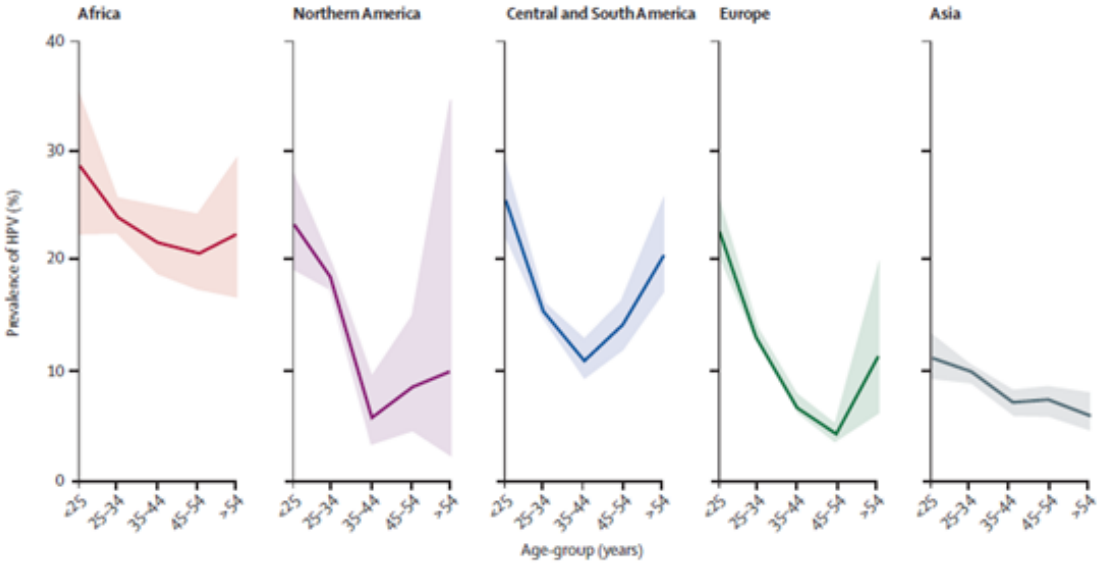


Figure 5.1: Age-specific HPV prevalence among women with normal cytology, by world region. (Shaded areas represent 95% CIs). Adapted from Sanjose et al., (2007).

Figure 5.1 shows age-related HPV prevalence estimates among women without cervical abnormalities, by world region (shaded areas represent 95% CIs) according to Sanjose *et al*., (2007). It is quite apparent from several studies such as these that the age-related trend of HPV infection varies slightly across age groups as it does from one geographic region to another (De Sanjosé *et al*., 2007; Bruni *et al*., 2010). Overall, the predominant pattern seems to portray an early peak, usually following the onset of sexual activity (Jacobs *et al*., 2000; Kjaer *et al*., 2001), followed a decline in HPV infections in middle age (Jacobs *et al*., 2000; Sellors *et al*., 2000; Matos *et al*., 2003; Munoz *et al*., 2004b) and then a variable pattern afterwards. U-shaped curves have also been reported, but there are discordant reports on the age group with the lowest prevalence. For instance, at ages 35–54 years in Costa Rica, (Herrero *et al*., 2000) and 35–44 years in Mexico (Lazcano-Ponce *et al*., 2001).

In three studies from sub-Saharan Africa (Serwadda et al, 1999; Castellsague et al, 2001; De Vuyst et al, 2003), the prevalence of HPV also declined with age and among older women in agreement with data from the present study (Figure 5.1).

The highest HR HPV prevalence was reported for women aged below 45 years and this finding is consistent with a higher number of recent sexual partners associated with this age group. Elsewhere, a similar observation was made in women younger than 30 years (Hibbitts *et al.*, 2006; de Sanjose *et al.*, 2007).

The elevated prevalence of viral infections in middle and old age in this study could have several plausible explanations. A fraction of men and women in Kumasi may continue to harbour multiple sexual contacts throughout their life and therefore re-infect themselves and their spouses. In our study, 79% of women older than 35 years have had more than one sexual partner. Women in this age bracket are the major patrons of extended-stay funerals which are notorious for promiscuous behaviour in this part of Ghana. Such women may also experience a diminished immune response to HPV infections, possibly due to the high rate of concomitant genital infections observed in this study. The reason for the second, menopausal peak (average age of menopause in the current study was 48.36 (95% CI: 47.33-49.40) could be attributed to one or more non-mutually exclusive mechanisms, such as reactivation of latent infections acquired earlier in life (Daud *et al.*, 2011), a gradual loss of type-specific immunity as a result of co-infection with HIV in populations endemic with HIV, or to acquisition of new infections due to sexual contacts with new partners later in life (Trottier and Franco, 2006). In this study only a third of HPV infected women above 64 years were married. Therefore, factors related to immunity may be more important in determining the burden of infection in this age group. Additional factors include low

vaginal secretions after menopause reducing vaginal lubrication and likelihood of microtears that allow HPV the opportunity to infect basal cells. Similarly, reduced secretions may result in low levels of protective viral neutralising antibodies in the vaginal environment again favouring HPV infection. In older women, reduced general immune status may result in delayed clearance or persistence of infection both of which may result in accumulation of infections and thus a high prevalence.

5.3 Overall distribution of HPV Genotypes

This study represents the largest effort to characterize the distribution of HPV genotypes among women in the Kumasi Metropolis. The data covers 500 women attending cervical screening at the three major centres in the metropolis over a two year period. Using a megasensitive nested multiplex PCR (NMPCR) assay that integrates degenerate E6/E7 consensus probes and type-specific probes for detecting, the prevalence of HPV oncogenic DNA, the frequency of high risk HPV genotypes present in decreasing order were HPV- 52, 56, 35, 18, 58, 68, 51, 39, 45, 16, 59, 33, 31. HPV 18 was found to be more prevalent than HPV 16. Low risk HPV were also detected in the following order: HPV 42, 43, 66, 6/11 and 44 (Tables 4.2b and 4.2c).

It is a common feature of cervical cancer to find HPV DNA firmly integrated into the cellular genome. This phenomenon may result in a breach in the genomic integrity of the circular viral genome which when found in the L1 gene, may prevent its detection by specific primers used to establish a prevalence estimate (Matzow *et al.*, 1998). In this study, the use of degenerate primers for E6 and E7 ORF allows for the detection of most viral

types even in those specimens in which the viral genome may have integrated into the host cell genome.

A few studies have reported HPV genotype distribution among the general population of Ghanaian women. Yar *et al.*, (2015) recently published findings from a pilot case-control study involving HIV women. The most common high-risk HPVs detected were 58, 35, 31, 68, 53, 52, 18, 16, etc.. The authors concluded that significant variations exist in HPV genotypes among HIV-infected and uninfected women. The high HIV prevalence could explain the observed HPV genotype distribution, since HIV-infected women are reported to acquire a broader spectrum of HPV genotypes compared to HIV-naïve women (Clifford *et al.*, 2006b; Didelot-Rousseau *et al.*, 2006; Sahasrabuddhe *et al.*, 2007).

An interesting finding of our study was the higher prevalence of HPV 18 compared to 16. This finding has been documented in many other available local studies both in normal cervixes (Brandful *et al.*, 2014; Yar *et al.*, 2015) and cancerous tissue (Attoh *et al.*, 2010; Awua *et al.*, 2016). In the study by Attoh *et. al.*, HPV 18 was found to be more prevalent than HPV 16 among Ghanaian women with cervical cancer- mostly adenocarcinomas, followed by HPV 45, 39, 35, 52 and 56. (Attoh *et al.*, 2010).

There is emerging concern about the possibility of certain HPV types being more common in Sub-Saharan African women than elsewhere. 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 colposcopically normal women 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). However, a low prevalence of HPV-16, HPV-53 and HPV-18 has been reported in the USA (Dunne *et al.*,

2011) and Greece (Stamataki *et al.*, 2010) similar to that of the present study.

Gravitt *et al*., (2002) postulated that type-specific HPV prevalence may be influenced by the type of assay used and by the preponderance of multiple HPV infection in certain populations. The former is particularly important because of cross-reactivity of certain HPV-types and is traceable to the sensitivity of assay. This position is however contentious since each study used a different assay and overall different assays will also mean varying sensitivities. Any biases introduced by individual assays should be expected to even out over several methods and not be reflected in the general picture. Again, in this study, we used a highly-sensitive nested multiplex assay that is intrinsically robust to false-positive results. In fact the proportion of HPV positive multiple infections were lower in this study (tables 4.6 and 4.7) compared to results from elsewhere (Cuschieri *et al.*, 2004b; Hibbitts *et al.*, 2006) corroborating our claim that the nested-PCR assay used is very robust to crossreactivity between viral genotypes.

Furthermore, studies from sub-Saharan Africa have shown variations in the relative ranking of HPV types that cannot be compatible with chance as some suggest. The sheer concentration of such findings better lends itself to the explanation that other factors such as HIV/AIDS endemicity might be able to shape the distribution of HPV types in Africa. If this hypothesis is true, only suitably designed studies among cohorts of HIV-diagnosed patients can actually tell. As it is, the relative fraction of HPV 16 and 18 in cervical cancer is beyond the full grasp of opportunistic population studies of this nature.

Meanwhile, although the likelihood of HPV 16 and 18 discovery with increasing severity of cervical findings is well noted (Clifford *et al.*, 2003), this study found that HPV 18 was more likely to be associated with a normal cytology result. As stated earlier, the reason for this disparity is not only unclear but also inconclusive. The type-specific distribution of HPV among 799 cervical cancer biopsies from Africa showed that HPV 16 accounted for 50.2% of samples, HPV 18 for 14.1%, and HPV 45 for 7.9% (i.e., a distribution similar to that found worldwide)(Clifford *et al*., 2003b).

5.4 Prevalence of low risk and high risk any type HPV in cervical pathology The prevalence of HR-HPV increased with increasing cytological grade. Remarkably, a significantly greater prevalence of HPV infection was seen among women with more severe cytology findings but the correlation between high-risk or low-risk HPV infection and severity of concurrent cytological findings among women was rather weak (Table 4.8). Possibly, an inability to transfer positive cells from sampling device to glass slide can result during sample preparation so they are not detected in cervical cytology. The resulting large false negative rate could undermine the correlation. However virus-infected positive cells not transferred may be washed off sampling device and made available for HPV detection and genotyping thus increasing the fraction of HPV-positive, cytology-negative samples.

The HPV genotype distribution in LSIL, ASCUS and in women with normal cytology was similar to that reported in previous studies (Kay *et al.*, 2003; Said *et al.*, 2009). However, the most prevalent high-risk genotypes in HSILs were HPV-16 and 52, which differ from previous studies conducted in sub-Saharan Africa and the rest of the world, where the most prevalent genotypes in HSILs were HPV-16 and 18 (Kay *et al.*, 2003; Bosch and Harper,

2006). In a study by Said *et al*., (2009) the most prevalent genotypes in HSILs were HPV35 followed by 58 and 66, and contrasts with previous study findings in South Africa by Kay *et al*., in Cape Town in which the most prevalent genotypes in HSILs were HPV-16 and 18. This difference could represent regional variations and differences in the populations studied. The present study population was women attending cervical screening, mostly in response to public education in churches and on radio and without any specific risk factors.

5.4.1 Risk of HR-HPV infection was doubled when cytological abnormalities were present

A major strength of the current study (apart from the sample size) is the availability of concurrent Pap smear and HPV results for most women making it possible to investigate the distribution of HPV in cervical pathology (Tables 4.5,4.6 and 4.7). There was a greater prevalence of HPV infection in women who had adverse cytological smears compared to those with normal smears. As expected, women who had abnormal Pap smear findings had a preponderance of HR HPV infection (Table 4.5). The prevalence of HR HPV infection was 59.3% among women with abnormal simultaneous pap result compared to 29.8% among women with normal Pap smear findings (Table 4.5). The commonest HPV types in abnormal cytology were HPV 18, 52, and 16/45/35/56 all in joint third position. Among women with normal cervixes, the commonest were HPV 52, 56, 35, 18, and 58/68 in decreasing order of prevalence. HR HPV conveys an increased risk of development of cervical neoplasia according to Bosch *et al*. (1995) and in this study HR HPV prevalence doubled in women with cytological abnormalities. Similar results have been previously documented in sub-Saharan Africa (Said *et al.*, 2009).

The oncogenic ability of HPV is based on the activity of E6 and E7 oncoproteins on cell cycle regulation, and has been demonstrated in several studies (Pim *et al*., 2012). High risk HPV types are notorious for their effective ability to transform the normal cell phenotype into a malignant one while the same cannot be said of low-risk types. Differences in E6 and E7 regulation exists between HR HPV and LR HPV and has been documented to affect levels of expression of the oncoproteins. The comparison of LR HPV and HR HPV E6 function show that both high-risk and low-risk types bind to p53 and E6AP, but only highrisk types have been shown to degrade p53 (Caldeira *et al.*, 2000; Akgül *et al.*, 2006). Other oncogenic E6 functions that are exclusive to HR HPV include immortalization of Rbinactivated human cells, inhibition of keratinocyte differentiation, telomerase activation, cmyc activation, and induction of genetic instability (Pim *et al.*, 2012). In addition, HR HPV E6 oncoprotein contains a C-terminal PDZ binding domain, which binds to and degrade multiple tumor suppressor proteins such as DLG1, MAGI-1, and Scribble, while all but a few LR HPV types lack this motif (Pfister, 2003).

There are several mechanisms by which E7 oncoprotein causes genomic instability and these are exclusive to HR HPV. The mechanism starts with binding and targeting Rb for ubiquitin-mediated degeneration, disrupting the Rb-E2F complex and releasing the E2F transcription factor. Unique protein domains of HR HPV E7 allows specific and highaffinity binding of additional members of the Rb family, p107 and p130 which are subsequently degraded. This activity is lacking in LR HPV E7 protein and because of its low affinity for Rb, family degradation occurs only in p130. The HR HPV E7 disruption of Rb-family complexes causes de-repression of many additional S-phase genes. Additional E7 functions that are present in HR HPV but lacking in LR HPV include activation of the

cfos and p73 promoters and STAT-1 suppression. Furthermore, HR HPV E7 but not LR HPV E7 is able to bypass DNA damage- or differentiation-mediated growth arrest. This difference can be attributed to the inability of LR HPV E7 to degrade Rb and its lower affinity for p21 abrogation, both required for growth arrest evasion (Pim *et al*., 2012).

Again, women with atypical pap smears had a greater odds ratio to harbour multiple HR HPV infections. This discrepancy was not apparent for women with typical pap smears. Women with abnormal cytology may depict greater sexual activity as compared to those with a normal cytology and could tend to harbour multiple HPV infections as a result (Sasagawa *et al.*, 2001; Cuschieri *et al.*, 2004a). Significant differences in the mean number of HPV types detected between cytologically normal and dysplastic samples has also been reported (Fife *et al.*, 2001). However, when severity of results are taken into consideration, no significantly higher risk of high grade cervical neoplasia (Cuschieri *et al.*, 2004a) or carcinoma (Rolón *et al.*, 2000) was found in women with multiple HPV infections compared with those who were infected with a single HPV type. It is thought that a high prevalence of multiple high risk human papillomavirus (HR-HPV) types in all grades of cervical neoplasia emphasises the lack of a cooperative carcinogenic relation between particular pairs or groups of HR-HPV types(Schmitt *et al.*, 2013a) and is thought by some to be an artefact of age-related sexual activity and reflects the common sexual transmission of multiple HR-HPV types together (Cuschieri *et al.*, 2004a).

5.5 Multiple human papillomavirus (HPV) infections and clustering patterns

In this study, using GP-E6-3F forward and two consensus back primers (GP-E7-5B and GP-E7-6B), multiple HPV genotypes were detected in about half (46%) of the HPV

positive samples (Table 4.11). Clustering of HPV types within women has been observed in previous HPV studies irrespective of the design or assays used (Thomas *et al.*, 2000a; Liaw *et al.*, 2001a; Rousseau *et al.*, 2001a; Chaturvedi *et al.*, 2005b; Méndez *et al.*, 2005; Vaccarella *et al.*, 2010). The maximum number of HPV sequences detected in a single woman was 5 and of the 18 HPV types screened, HPV-6/11, HPV-33, HPV-44, HPV-39, HPV-58 and HPV-45 had the highest risk estimates to cluster with other HPV types (Table 4.12).

The phenomenon of multiple HPV infections are involved in a vast fraction of HPVpositive women, an observation that has been made in most previous studies (Castellsague *et al.*, 2001; Gravitt *et al.*, 2002; De Vuyst *et al.*, 2003). In a 2014 study by Brandful *et al*., among women of reproductive age, frequency of multiple infections was high (56.7%) compared to the present study. In general, that study had a high rate of low risk HPV genotypes (42 and 43) compared to this study. The variation in the rates of multiple infections can be attributed to diversity of populations studied: while that study was restricted to women in reproductive years, this study also included older women who may have lower rates of multiple infections (Cuschieri *et al.*, 2004a). Data from neighbouring countries have shown similar high frequencies of multiple-infections. A 52.9% rate of multiple infection was observed in a study in Burkina Faso (Didelot-Rousseau *et al.*, 2006), while a 40.2% rate was reported by a study in Benin (Piras *et al.*, 2011) and 33.5% of infections in a Nigerian study involved more than one HPV type (Thomas *et al.*, 2004). These may suggest a high rate of multiple-infections among the general population of women in the West African region. Also, it is believed that in general, PCR systems using multiple primers are more robust for detecting multiple infections than systems using single consensus primers

(Clifford *et al.*, 2006a). Further studies reporting on the phenomenon of multiple HPV infections among the women with normal cervical findings would greatly clarify the reason for the elevated rate of multiple infections observed in this particular population.

Differences in study power and processing methods may be another vital reason for disparity in estimates of multiple infections, highlighting the need for using standard protocol and large sample size to enhance comparability of HPV data. Among confirmed cancer studies in Ghana, multiple infection rates from 26% to 52 % have been reported. The smallest estimate of multiple HPV infection in Ghana was reported by a pilot retrospective study to determine HPV genotypes prevalent in Ghanaian women with cervical cancer: Attoh *et al.*, reported that multiple infections were detected in 13 (26%) of the 50 samples selected, the most common co-infections being HPV types 16/18 (18%). Using a larger sample, Awua and colleagues have investigated multiple HPV infections among confirmed cervical cancer cases diagnosed at the Korle-Bu Teaching Hospital during the period January 2004 to December 2006 (Awua *et al.*, 2016). They found a 52.2% prevalence of multiple HPV infection and HPV18, HPV59, HPV45 were the most likely to be found in multiple infections (Awua *et al.*, 2016). Data from a multicentre study conducted in women from Ghana, Nigeria and South Africa, who had cervical lesions clinically suggestive of

ICC gives an overall multiple infection rate of 11.1% and 19.0% for Ghana in particular (Denny *et al.*, 2014).

To understand viral interactions and the cross-reactivity of natural or vaccine-induced responses, it is desirable to investigate whether multiple human papillomavirus (HPV) infections, particularly certain combinations of types, have the tendency to cluster. Few

studies, however, have been able to evaluate pair-wise clustering across a range of individual HPV types. Thomas *et al.* (2000a) evaluated HPV6, 11, 16, 18, 31, and 45 among 518 female university students in the United States using MY09/11 primers. No two-type infections were more or less likely than any other combination. Chaturvedi *et al*. (2005b) also focused on the clustering of 27 HPV types in multiple infections among 854

HIV-negative and 275 HIV-positive women from the United States, but they used α species rather than individual HPV types as the unit of their analyses. In a study of 1,610 Colombian women who were tested using GP5+/6+ with subsequent EIA genotyping method, Mendez et al. (2005) reported an excess of clustering for several two-type combinations (including HPV 33 and 58, HPV 33 and 39, and HPV 18 and 45, after adjustment for age and lifetime sexual partners. In 2010, Vaccarella *et al.*, conducted a large multi-centrer study which included approximately 14,000 women, of whom 1,720 were HPV-positive and 554 (32.2%) had multiple HPV infections, using a common protocol in all centres that allowed a systematic investigation of multiple infections with specific twotype combinations among the 15 most common HPV types. Among combinations of

specific HPV types, the tendency to cluster increased with the genetic similarity of the L1 region. A higher-than-chance tendency to cluster was found for closely homologous types, including HPV33/58, 18/45, 33/35, and 31/35. An excess of multiple infections, however, was observed only when enzyme immunoassay, and not reverse line blot, was used as the genotyping method. Thus, the different results by genotyping method suggest that the apparent clustering of HPV infections was an artifact of the measurement process and hint that HPV infections are perhaps independent phenomena (Vaccarella *et al.*, 2010). In

another report, no significant difference in odds ratio was seen for infection with multiple HPV types compared to infection with a single type, although the highest odds ratio reported was for multiple-type infections that include HPV16 (Munoz *et al.*, 2003b). Crosshybridization of DNA typing probes has been cited as a possible explanation for high rates of multiple infections and an assay-specific artificial preponderance of certain clustering patterns (Vaccarella *et al.*, 2010). Cross-hybridization might occur when the DNA probe designed to match a specific DNA sequence hybridizes with homologous sequences from another HPV type, leading to the apparent detection of two HPV types when only one is present (Vaccarella *et al.*, 2010).

5.6 Risk factors for abnormal cervical findings

The current study also sought to examine the correlates of abnormal cytology and their implications for disease prevention among women in the Kumasi metropolis (Tables 4.13 and 4.14. As is commonly reported, older women (>40 years old) had significantly increased odds to produce abnormal cervical cytology compared to younger women (Table 4.13). This finding is further confirmation of the widely accepted belief that 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). 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). In a persistent infection lasting several years, overexpression of viral oncogenes lead to abrogation of various cell-cycle regulatory mechanisms and accumulation of a host of other pre-cancerous cell-level events that eventually lead to an altered phenotype. Since these changes occur over time, it is plausible

that the incidence of cervical lesions may be higher in older women than in younger women who have a greater fraction of freshly acquired, more benign, infections.

Abortion status had significant effects for predicting cervical dysplasia (Table 4.14). Compared to abortion-naïve women, women who had had even one aborted pregnancy had higher odds for abnormal cervical cytology. This odds increased with increasing number of abortions. Possibly, multiple abortions may generate significant trauma to cervical epithelium and consequent microtears that increase the probability of HPV access to basal cells to initiate an infection. Again, high induced abortion rates may tell of a woman's more liberal sexual lifestyle and practice of high risk sexual behaviour that can increase her odds for a persistent HPV infection.

Having concurrent multiple HPV infection was associated with a significantly higher prevalence odds ratio for Pap smear abnormality, 0R=4.4 (95% CI: 1.4-14.0) compared to single infection status (Table 4.14). The presence of multiple HPV genotypes may suggest repetitive exposure to multiple HPV genotypes due to high-risk sexual behaviour (Said *et al.*, 2009). A decade ago, besides an established role for certain human papillomavirus (HPV) genotypes in the aetiology of cervical cancer, little was known about the influence of multiple-type HPV infections on cervical lesion risk. It was believed that co-infections with multiple HPV types could synergistically multiply risk in cervical carcinogenesis (Trottier *et al.*, 2006). However, in an experimental study of multiple co-infection with HR HPV types, it was decisively shown by micro-dissection followed by in-situ hybridisation using multiple chromogens that each infecting type is responsible for specific areas within the lesion and no two viruses infect the same area within the lesion leading to the axiom: one virus, one lesion (Quint *et al.*, 2012).

Compared to nulligravidate women, the odds ratio for Pap smear abnormality increased as number of conceived pregnancies but this difference was not up to the level of statistical significance (Table 4.15). Studies reporting young age at sexual debut as a significant independent factor for cervical lesions were among younger women who also a lower mean age at first sexual experience. The relationship between young age at sexual debut may be more recognisable among younger cohorts such as school girls within a clear age-bracket than among women of more varied ages. It is possible that older women may misrepresent their real age at sexual debut as a result of a more distant memory of the exact date compared to younger women for whom the memory will be relatively recent. Further studies may be required to prove this hypothesis.

5.7 Risk factors for HPV infection

The current study also sought to examine the correlates of genital HPV infection and their implications for disease prevention. Age, educational status, marital status and ethnic grouping were not significant independent predictors of HPV positivity. There were no significant trends in HPV prevalence for different numbers of birth, pregnancies or abortions (Tables 4.16 and 4.17). However, HPV positivity was significantly associated with sexual history variables of women: multiple lifetime sex partners and having a husband with unmarried (extramarital) partners were independent significant predictors of HPV infection among women. In another Ghanaian study involving a cross-section of 75 women selected from the gynaecology outpatient clinic of the Korle-Bu Teaching Hospital, Accra, reporting more than three lifetime sexual partners was an independent determinant of HPV status as was illiteracy (Domfeh *et al.*, 2008).

A lot of previous studies have showed that the main risk factor for HPV infection was the number of sexual partners (Bauer *et al.*, 1993; Burk *et al.*, 1996; Kjaer *et al.*, 1997; Stamataki *et al.*, 2010). Not only is sexual intercourse regarded as the primary route of genital HPV infection, there is also strong and consistent association between lifetime numbers of sexual partners and HPV prevalence in women (Koutsky and Kiviat, 1999; Winer and Koutsky, 2004). There are also reports stating that single women also have an increased risk of HPV acquisition from sporadic or serial relationships with new and recent sexual partners (Winer and Koutsky, 2004). Although not a significant association in this study, in another study in Nigeria, single women were at statistically significant odds of 2.1 for having a prevailing HPV infection, as opposed to married women or widowed and/or divorced women (Thomas *et al.*, 2004). Stamataki et al., (2010) reported findings on HPV infection among Greek women aged 16 to 45 years attending a gynecological outpatient clinic. Univariate logistic analysis showed significant associations between HPV infection and age, monthly income, marital status, number of full term pregnancies, number of sexual partners, smoking status, and alcohol consumption. No significant relationships were found between HPV infection and educational level, nationality, methods of contraception, age at first sexual intercourse, and history of previous HPV infection or other sexual transmitted diseases (Stamataki et al., 2010).

Remarkably, age was not a significant risk factor for HPV positivity in the current study (Table 4.16). One reason may be the inclusion of post-menopausal women in the current study. Studies reporting significant associations with age are usually comprised of only premenopausal women up to age 40 (Peyton *et al.*, 2001; Stamataki *et al.*, 2010). Additionally, younger age at sexual debut was not significantly associated with greater

likelihood of HPV infection. The average duration between the onset of menses and initiation of sexual intercourse among HPV positive women was approximately 2 years less than HPV negative women (data not shown). Several cross-sectional studies have reported that earlier sexual debut or shorter intervals between menarche and sexual debut are risk factors for prevalent HPV infection (Kahn *et al.*, 2002). However, the reasons for this relationship are unclear. Possibly earlier age at onset of intercourse may be a marker for other risky sexual behaviour, such as greater lifetime numbers of partners and concurrent partnerships (Aral and Holmes, 1999) in some cultures. Indeed, one study has reported that the association of

HPV-DNA acquisition with age at first intercourse is mediated by other sexual behaviour variables (Kahn *et al.*, 2002).

It is noteworthy that polygamous sexual relationships and poor perception of husbands' fidelity were common in this population, and significant predictors of HPV positivity as well (Table 4.18).

Chapter 6 CONCLUSIONS

The present study found that the prevalence of HPV infection (of any type) among women presenting for screening in Kumasi was 37.6% (Table 4.2a). According to global and regional estimates, this prevalence is high and demands immediate public health intervention. All 18 HPV types (five low-risk and thirteen high-risk HPV genotypes) screened were identified among the population sampled. The prevalence of high-risk, and low-risk HPV types was 31.4% and 14.2%, respectively.

Tables 4.2b and 4.2c also shows the distribution of HPV types detected among the general women population presenting for screening in the Kumasi metropolis. The commonest HR types were, HPV-52 (11.6%; 58 women), HPV-56 (7%; 35 women), HPV-35 (5%; 25 women), HPV-18 (4.8%; 24 women), HPV-66 (3.8%; 19 women), and HPV-58 (3.2%; 16 women). HPV 42, a low-risk type, was also common (7.8%; 39 women). The frequency of HPV 16 detection was 1.4% (7 women). Overall, the prevalence of HPV infection among women who responded to the call for screening in Kumasi was high.

Approximately seventeen percent (31/186) of women infected with HPV were found to have genotypes 16 and/or 18. Therefore, an important conclusion from the present study is that HPV16 and HPV18 are under-represented in women with normal cytology by comparison with their importance in populations with confirmed / suspected cervical lesions in which the HPV 16/18 fraction is in excess of 70%.

The high prevalence of HPV types generally regarded as high-risk types is an important finding of this work (Tables 4.2a and 4.2b). The overall prevalence of HR HPV was 31.4% (95% CI: 27.4 – 35.7), (84.4% of HPV positive cases) compared to 14.2% (95%

CI: 11.3 – 17.6), (38.2% of HPV positive cases) for LR HPV types. In general, 22.6% of all HPV infections were hybrid infections involving both HR and LR types, 61.8% involved only HR types and 15.6% involved only LR types.

Cytological abnormalities ranging from LSIL to SCC were present in 6.4% of the total study population (Table 4.1). Out of a total 592 slides evaluated, 8 (1.4%) showed atypical squamous cells of undetermined significance (ASCUS), 9 (1.4%) low-grade squamous intraepithelial lesions, and 2 (0.3%) high-grade squamous intraepithelial lesions. Additionally, 3 women had squamous cell carcinoma. None of the women was found to have atypical glandular cells or adenocarcinoma. Other conditions including infections were detected among the population. Among all women screened, 43 had non-specific cervicitis (7.3%), 12 had candidiasis alone (2%), 12 had bacterial vaginosis alone (2%) and 1 had *Trichomonas vaginalis* (0.2%) infection.

In this study, women who had abnormal pap smear findings were also significantly more likely to be infected with the human papillomavirus, and therefore suggests that viral onco-proteins have a major role to play in the manifestation of cervical epithelial abnormalities in this region. Among women with normal cytology, the prevalence of HPV was 35.7% and among women with abnormal cervical findings it was 62.9% (Table 4.5).

Furthermore, HR HPV prevalence in women with cytological abnormalities was double the rate found among women with normal cervical smears. Specifically, the prevalence of HR HPV infection was 59.3% among women with abnormal simultaneous pap result compared to 29.8% among women with normal pap smear findings. This significant finding goes to further support the role of high-risk HPV E6 and E7 oncogenes in festering cervical dysplasia in the current population (Table 4.5).

Across the spectrum of cervical disease, HPV prevalence significantly increased with severity of cervical lesions (Table 4.8).

The commonest HPV genotypes detected among women with ASCUS were HPV-18, 52 and 68. Among women with LSIL most prevalent were HPV-52, HPV-18 and HPV 45. Additionally, HPV-16 and 52 were the most frequent genotypes in HSILs and SCC (Table 4.10) confirming geographical variation in HPV genotype distribution. This might be an indicator of the potential success of the currently available HPV vaccines, especially the expanded panel vaccine (9vHPV vaccine/ Gardasil 9), in this region. Further studies on the natural history of HPV 52 infection should give better insight on the oncogenic potential of this type in the population and answer curious questions raised by the current study.

Among HPV positive women, 46% had pluralistic infection with multiple HPV genotypes. The maximum number of HPV sequences detected in a single woman was 5 out of the 18 HPV types screened (Table 4.11). HPV-6/11, HPV-33, HPV-44, HPV-39,

HPV-58 and HPV-45 had the highest risk estimates to cluster with other HPV types. HPV-16 and 18 however, were not likely to be associated with multiple infection status. (Table 4.12). Again, women with atypical pap smears had a greater tendency to harbour multiple HR HPV infections. This discrepancy was not apparent for women with typical pap smears. In general, the present study found that the main oncogenic HPV types did not show a greater predilection for multiple infections in the context of the general population alone.

Old age and a woman's previous history of abortions were the main correlates of abnormal cytology among study women. The study realized that older women (>40

years old) had significantly increased odds (OR=3.0; 95% CI: 1.3-6.8) for abnormal cervical findings (Tables 4.13 and 4.14). Also, compared to abortion-naïve women, women who had had even one aborted pregnancy had higher odds for abnormal cervical cytology 0R=4.2 (95% CI: 0.9 – 20.0). Having a reproductive history of at least 2 abortions increased the odds further to 6.5 (95% CI: 1.5 – 28.2). A woman's level of education, marital status and ethnicity were not significant predictors of cytology outcome. Moreover, number of pregnancies, young age at menarche, coitache and first pregnancy did not affect the odds of cervical disease (Table 4.14). Having concurrent multiple HPV infection was associated with a significantly higher prevalence odds ratio for Pap smear abnormality, OR=4.4 (95% CI: 1.4-14.0) compared to single infection. Additionally, simultaneous infection with both 9-valent-vaccine-preventable HPV genotypes and other HPV types excluded from the vaccine had higher prevalence odds ratio for Pap smear abnormality OR=4.1 (95% CI: 1.3-13.9) (P=0.042), compared to having only 9-valent vaccine types, OR=1.6 (95% CI: 0.3-7.3). For married women, the age of sexual partner had a significant relationship with her Pap smear result (P=0.026) as well (Table 4.15).

HPV infected women were significantly older than non-infected women. Among the sexual history variables studied, having a history of multiple sexual partners (P=0.048) and extramarital activity of woman's partner (P=0.029) were the main correlates of HPV infection among women (Table 4.4, 4.16 and 4.17). In fact, having multiple lifetime sexual partners was associated with an OR of 1.7 (95% CI: 1.1-2.9) for an HPV infection compared to women with single lifetime partners (P=0.049). However a woman's age at coitache, polygamy, marital history of woman's partner and main partner's age were all not significantly associated with HPV infection status in this

population. This study also detected that women who have a habit of vaginal washing or douching do not have an increased risk of HPV.

In sum, using a highly sensitive and specific nested-multiplex PCR assay, this study successfully estimated the prevalence and distribution of genital HPV genotypes in a representative population of Ghanaian women in the Kumasi Metropolis, Ashanti Region, Ghana. The study provides descriptive statistics on the extent of multiple human papillomavirus (HPV) infections, and inferential statistics on which combinations of high-risk types have the tendency to cluster together are also presented and discussed. Cervical abnormalities were classified and the association of HPV types evaluated for various degrees of cervical dysplasia as well. Finally the correlates of abnormal cervical findings in the study population are also discussed. Therefore this study fulfils the objective of garnering baseline data for future analyses in this population to monitor vaccination programme effects on the prevalence of the vaccine targeted HR types 16 and 18 for Cervarix or Gardasil or the expanded panel for Gardasil-9, the proportion of cross-protection provided to non-vaccine HR HPV types and the issue of type-replacement.

6.1 LIMITATIONS

The relatively high proportion of inadequate cervical cell samples was undesirable. This may be due to problems in the preparation or storage of cervical smears or to the limited experience of our study nurses in cervical cell collection.

Despite the use in this study of a series of primers that recognize numerous types of HPV, no primers have been known to be able to recognize all 200 sequenced genotypes or the approximately 40 alpha-papillomaviruses capable of infecting the human

anogenital tract. This limitation must be taken into consideration when interpreting our data, and we must also consider the possibility that these viral genotypes, which have not yet been sequenced and are not detectable with the primers presently available, may be prevalent in the population as well.

6.2 RECOMMENDATIONS

Cervical cancer and precursor epithelial cell abnormalities are still common among women in Kumasi for a disease that can be prevented by early detection through routine screening and management. This study provides adequate background data to make the implementation of cervical cancer screening in all eligible women a national healthcare priority in Ghana.

Future work should incorporatee HPV genotyping assays into cervical screening programmes to form a basis for HPV type-specific surveillance to monitor the efficacy of prophylactic vaccination.

This study highlights the need for a national policy for vaccination. and recommends that the Ghana Health Service evaluates the nonavalent vaccine for approval and use. However, a bigger nationwide study with even greater statistical power is strongly recommended.

Further studies on the natural history of HPV 52 infection should give better insight on the oncogenic potential of this type in the population and answer curious questions raised by the current study.

In general, the present study found that the main oncogenic HPV types did not show a greater predilection for multiple infections in the context of the general population

alone. It would be interesting to repeat the experiment in a larger cohort of women with abnormal cervical findings.

Finally, HPV screening in low-skill settings such this may be improved by employing more efficient liquid based cytology methods to minimize the problem of specimen inadequacy and sampling error.

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.

ACS (2010) Cervical Cancer: Prevention and Early Detection. In *http://www.cancer.org/acs/groups/cid/documents/webcontent/003167-pdf.pdf* [A.C. Society, editor.

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

Adjorlolo-Johnson G., Unger E.R., Boni-Ouattara E., Touré-Coulibaly K., Maurice C., Vernon S.D., Sissoko M., Greenberg A.E., Wiktor S.Z. and Chorba T.L. (2010) Assessing the relationship between HIV infection and cervical cancer in Cote d'Ivoire: a case-control study. *BMC infectious diseases* 10, 1.

Agoff S.N., Lin P., Morihara J., Mao C., Kiviat N.B. and Koutsky L.A. (2003) p16(INK4a) expression correlates with degree of cervical neoplasia: a comparison with Ki-67 expression and detection of high-risk HPV types. *Mod Pathol* 16, 665-673.

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.

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 typespecific persistence of human papillomavirus in human immunodeficiency virus (HIV)-positive and HIV-negative women. *Journal of Infectious Diseases* 184, 682690.

Akgül B., Cooke J.C. and Storey A. (2006) HPV-associated skin disease. *The Journal of pathology* 208, 165-175.

Albert S., Oguntayo O. and Samaila M. (2012) Comparative study of visual inspection of the cervix using acetic acid (VIA) and Papanicolaou (Pap) smears for cervical cancer screening. *ecancermedicalscience* 6.

Almonte M., Gonzales M., Delgado J.M., Cuzick J., Luciani S. and Sasieni P. (2011) RISK FACTORS FOR HIGH-GRADE CERVICAL LESIONS IN THE TATI PROJECT. *27th International Papillomavirus Conference and Clinical Workshop*, 03:18.

ALTS (2003) ASCUS-LSIL Triage Study (ALTS) Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 188, 1383-1392.

Anttila A., Kotaniemi-Talonen L., Leinonen M., Hakama M., Laurila P., Tarkkanen J., Malila N. and Nieminen P. (2010) Rate of cervical cancer, severe intraepithelial neoplasia, and adenocarcinoma in situ in primary HPV DNA screening with cytology triage: randomised study within organised screening programme. *BMJ* 340, c1804.

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., Castellsague X., De Sanjose S., Bruni L., Saraiya M., Bray F. and Ferlay J. (2011) Worldwide Burden of Cervical Cancer in 2008. *Annals of oncology* 22, 26752686.

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

ASCCP (2012) Invasive cancer of the cervix, pp. Website of the American Society for Colposcopy and Cervical Pathology.

Attoh S., Asmah R., Wiredu E.K., Gyasi R. and Tetteh Y. (2010) Human Papillomavirus Genotypes in Ghanaian Women with Cervical Carcinoma. *East African medical journal* 87, 4-8.

Awua A., Sackey S., Osei Y., Asmah R. and Wiredu E. (2016) Prevalence of human papillomavirus genotypes among women with cervical cancer in Ghana. *Infectious agents and cancer* 11, 1.

Baay M., Quint W., Koudstaal J., Hollema H., Duk J.M., Burger M., Stolz E. and Herbrink P. (1996) Comprehensive study of several general and type-specific primer pairs for detection of human papillomavirus DNA by PCR in paraffinembedded cervical carcinomas. *Journal of Clinical Microbiology* 34, 745-747.

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, 481485.

Band V., Zajchowski D., Kulesa V. and Sager R. (1990) Human papilloma virus DNAs immortalize normal human mammary epithelial cells and reduce their growth factor requirements. *Proc Natl Acad Sci U S A* 87, 463-467.

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

Bauer H.M., Hildesheim A., Schiffman M.H., Glass A.G., Rush B.B., Scott D.R., Cadell D.M., Kurman R.J. and Manos M.M. (1993) Determinants of genital human papillomavirus infection in low-risk women in Portland, Oregon. *Sex Transm Dis* 20, 274-278.

Bauer H.M., Ting Y., Greer C.E., Chambers J.C., Tashiro C.J., Chimera J., Reingold A. and Manos M.M. (1991) Genital human papillomavirus infection in female university students as determined by a PCR-based method. *JAMA* 265, 472-477.

Beausejour C.M., Krtolica A., Galimi F., Narita M., Lowe S.W., Yaswen P. and Campisi J. (2003) Reversal of human cellular senescence: roles of the p53 and p16 pathways. *The EMBO journal* 22, 4212-4222.

Bell J. and Waters S. (2014) *Doing Your Research Project: A guide for first-time researchers*: McGraw-Hill Education (UK).

Bernard H.U. (2002) Gene expression of genital human papillomaviruses and considerations on potential antiviral approaches. *Antivir Ther* 7, 219-237.

Bernard H.U., Chan S.Y., Manos M.M., Ong C.K., Villa L.L., Delius H., Peyton C.L., Bauer H.M. and Wheeler C.M. (1994) Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. *Journal of Infectious Diseases* 170, 1077-1085.

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.X. (2003) Epidemiology of human papillomavirus infections: new options for cervical cancer prevention. *Salud Publica Mex* 45 Suppl 3, S326-339.

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. and de Sanjose S. (2007) The epidemiology of human papillomavirus infection and cervical cancer. *Dis Markers* 23, 213-227.

Bosch F.X. and De Sanjosé S. (2002) 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., Manos M.M., Muñoz N., Sherman M., Jansen A.M., Peto J., Schiffman M.H., Moreno V., Kurman R. and Shan K.V. (1995c) Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. *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.

Bosch X. and Harper D. (2006) Prevention strategies of cervical cancer in the HPV vaccine era. *Gynecol Oncol* 103, 21-24.

Brandful J., Bonney E., Asmah R. and Apea-Kubi K. (2014) Oncogenic human papillomavirus (HPV) in women from Ghana. *Journal of Cancer Research and Experimental Oncology* 6, 31-38.

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.

Bulkmans N.W., Berkhof J., Bulk S., Bleeker M.C., van Kemenade F.J., Rozendaal L., Snijders P.J. and Meijer C.J. (2007a) High-risk HPV type-specific clearance rates in cervical screening. *British Journal of Cancer* 96, 1419-1424.

Bulkmans N.W., Berkhof J., Rozendaal L., van Kemenade F.J., Boeke A.J., Bulk S., Voorhorst F.J., Verheijen R.H., van Groningen K., Boon M.E., Ruitinga W., van Ballegooijen M., Snijders P.J. and Meijer C.J. (2007b) Human papillomavirus DNA testing for the detection of cervical intraepithelial neoplasia grade 3 and cancer: 5-year follow-up of a randomised controlled implementation trial. *The Lancet* 370, 1764-1772.

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

Burk R.D., Kelly P., Feldman J., Bromberg J., Vermund S.H., DeHovitz J.A. and Landesman S.H. (1996) Declining prevalence of cervicovaginal human papillomavirus infection with age is independent of other risk factors. *Sex Transm Dis* 23, 333-341.

Caldeira S., de Villiers E.-M. and Tommasino M. (2000) Human papillomavirus E7 proteins stimulate proliferation independently of their ability to associate with retinoblastoma protein. *Oncogene* 19, 821-826.

Castellsague X., Diaz M., de Sanjose S., Munoz N., Herrero R., Franceschi S., Peeling R.W., Ashley R., Smith J.S., Snijders P.J., Meijer C.J. and Bosch F.X. (2006) Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: implications for screening and prevention. *Journal of the National Cancer Institute* 98, 303-315.

Castellsague X., Menendez C., Loscertales M.P., Kornegay J.R., dos Santos F., GomezOlive 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., Fetterman B., Cox J.T., Shaber R., Poitras N., Lorey T. and Kinney W. (2010) The age-specific relationships of abnormal cytology and human papillomavirus DNA results to the risk of cervical precancer and cancer. *Obstetrics and Gynecology* 116, 76-84.

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.

Castle P.E., Schiffman M., Gravitt P.E., Kendall H., Fishman S., Dong H., Hildesheim A., Herrero R., Bratti M.C., Sherman M.E., Lorincz A., Schussler J.E. and Burk R.D. (2002) Comparisons of HPV DNA detection by MY09/11 PCR methods. *J Med Virol* 68, 417-423.

Castle P.E., Solomon D., Wheeler C.M., Gravitt P.E., Wacholder S. and Schiffman M. (2008) Human papillomavirus genotype specificity of hybrid capture 2. *Journal of Clinical Microbiology* 46, 2595-2604.

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

Chan S.-Y., Delius H., Halpern A.L. and Bernard H.-U. (1995) Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. *Journal of virology* 69, 3074-3083.

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. (2005a) Prevalence of human papillomavirus genotypes in women from three clinical settings. *J Med Virol* 75, 105-113.

Chaturvedi A.K., Myers L., Hammons A.F., Clark R.A., Dunlap K., Kissinger P.J. and Hagensee M.E. (2005b) Prevalence and clustering patterns of human

papillomavirus genotypes in multiple infections. *Cancer Epidemiology Biomarkers & Prevention* 14, 2439-2445.

Chen G., Hill A., Seffah J., Darko R., Anarfi J. and Duda R. (2005) Epidemiology of cervical cancer and dysphasia in a cross-sectional of women in Accra, Ghana. *Int J Trop Med* 1, 7-11.

Choo K.B., Lee H.H., Pan C.C., Wu S.M., Liew L.N., Cheung W.F. and Han S.H. (1988) Sequence duplication and internal deletion in the integrated human papillomavirus type 16 genome cloned from a cervical carcinoma. *Journal of Virology* 62, 1659-1666.

CIA (2010) World Fact Book: Ghana. .

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., 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 - 73.

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

Clifford G.M., Rana R.K., Franceschi S., Smith J.S., Gough G. and Pimenta J.M. (2005c) Human papillomavirus genotype distribution in low-grade cervical lesions: comparison by geographic region and with cervical cancer. *Cancer Epidemiology Biomarkers & Prevention* 14, 1157-1164.

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

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.

Cox J.T., Lorincz A.T., Schiffman M.H., Sherman M.E., Cullen A. and Kurman R.J. (1995) Human papillomavirus testing by hybrid capture appears to be useful in triaging women with a cytologic diagnosis of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 172, 946-954.

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., Cubie H., Whitley M., Seagar A., Arends M., Moore C., Gilkisson G. and McGoogan E. (2004a) Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population. *J Clin Pathol* 57, 68-72.

Cuschieri K.S., Whitley M.J. and Cubie H.A. (2004b) 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., Arbyn M., Sankaranarayanan R., Tsu V., Ronco G., Mayrand M.H., Dillner J. and Meijer C.J. (2008) Overview of human papillomavirus-based and other novel options for cervical cancer screening in developed and developing countries. *Vaccine* 26 Suppl 10, K29-41.

Cuzick J., Sasieni P. and Singer A. (1996) Risk factors for invasive cervix cancer in young women. *European Journal of Cancer* 32, 836-841.

Cuzick J., Szarewski A., Terry G., Hanby A., Maddox P., Anderson M., Steele S., Guillebaud J. and Kocjean C. (1995) Human papillomavirus testing in primary cervical screening. *The Lancet* 345, 1533-1536.

Danos O., Katinka M. and Yaniv M. (1982) Human papillomavirus 1a complete DNA sequence: a novel type of genome organization among papovaviridae. *The EMBO journal* 1, 231.

Daud, II, Scott M.E., Ma Y., Shiboski S., Farhat S. and Moscicki A.B. (2011) Association between toll-like receptor expression and human papillomavirus type 16 persistence. *International journal of cancer* 128, 879-886.

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

Davey D.D., Neal M.H., Wilbur D.C., Colgan T.J., Styer P.E. and Mody D.R. (2004) Bethesda 2001 implementation and reporting rates: 2003 practices of participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology. *Archives of pathology & laboratory medicine* 128, 1224-1229.

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

de Roda Husman A.M., Walboomers J.M., van den Brule A.J., Meijer C.J. and Snijders P.J. (1995) The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *Journal of General Virology* 76 (Pt 4), 1057-1062.

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., 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 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., 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. *Sexually Transmitted Diseases* 30, 137-142.

Dehn D., Torkko K.C. and Shroyer K.R. (2007) Human papillomavirus testing and molecular markers of cervical dysplasia and carcinoma. *Cancer* 111, 1-14.

Denny L., Adewole I., Anorlu R., Dreyer G., Moodley M., Smith T., Snyman L., Wiredu E., Molijn A. and Quint W. (2014) Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa. *International journal of cancer* 134, 1389-1398.

Denton K.J., Bergeron C., Klement P., Trunk M.J., Keller T. and Ridder R. (2010) The sensitivity and specificity of p16(INK4a) cytology vs HPV testing for detecting high-grade cervical disease in the triage of ASC-US and LSIL pap cytology results. *Am J Clin Pathol* 134, 12-21.

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.

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.

Dockter J., Schroder A., Hill C., Guzenski L., Monsonego J. and Giachetti C. (2009) Clinical performance of the APTIMA HPV Assay for the detection of high-risk HPV and high-grade cervical lesions. *Journal of clinical virology* 45 Suppl 1, S55-61.

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.

Doorbar J. (2005) The papillomavirus life cycle. . *Journal of clinical virology* 32, S7–S15.

Duensing S., Duensing A., Crum C.P. and Munger K. (2001) Human papillomavirus type 16 E7 oncoprotein-induced abnormal centrosome synthesis is an early event in the evolving malignant phenotype. *Cancer Res* 61, 2356-2360.

Duensing S. and Munger K. (2004) Mechanisms of genomic instability in human cancer: insights from studies with human papillomavirus oncoproteins. *International journal of cancer* 109, 157-162.

Dunne E.F., Sternberg M., Markowitz L.E., McQuillan G., Swan D., Patel S. and Unger E.R. (2011) Human papillomavirus (HPV) 6, 11, 16, and 18 prevalence among females in the United States—National Health And Nutrition Examination Survey, 2003–2006: opportunity to measure HPV vaccine impact? *Journal of Infectious Diseases* 204, 562-565.

Durowade K., Osagbemi G., Salaudeen A., Musa O., Akande T., Babatunde O., Raji H., Okesina B., Fowowe A. and Ibrahim O. (2012) Prevalence and risk factors of cervical cancer among women in an urban community of Kwara State, north central Nigeria. *Journal of preventive medicine and hygiene* 53.

Durst M., Croce C.M., Gissmann L., Schwarz E. and Huebner K. (1987a) Papillomavirus sequences integrate near cellular oncogenes in some cervical carcinomas. *Proc Natl Acad Sci U S A* 84, 1070-1074.

Durst M., Dzarlieva-Petrusevska R.T., Boukamp P., Fusenig N.E. and Gissmann L. (1987b) Molecular and cytogenetic analysis of immortalized human primary keratinocytes obtained after transfection with human papillomavirus type 16 DNA. *Oncogene* 1, 251-256.

Durst M., Gissmann L., Ikenberg H. and zur Hausen H. (1983) A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc Natl Acad Sci U S A* 80, 3812-3815.

Dyson N., Howley P.M., Munger K. and Harlow E. (1989) The human papilloma virus16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science* 243, 934-937.

El-Ghobashy A., Shaaban A., Herod J. and Herrington C. (2005) The pathology and management of endocervical glandular neoplasia. *International Journal of Gynecological Cancer* 15, 583-592.

Evander M., Edlund K., Boden E., Gustafsson A., Jonsson M., Karlsson R., Rylander E. and Wadell G. (1992) Comparison of a one-step and a two-step polymerase chain reaction with degenerate general primers in a population-based study of human papillomavirus infection in young Swedish women. *Journal of Clinical Microbiology* 30, 987-992.

FDA (2014) Approval letter—GARDASIL 9. Silver Spring, MD: Food and Drug Administration

Feichter G. and Meisels A. (2002) Task force consensus report on HPV-related changes of the lower female genital tract. *Acta Cytol* 46, 630-632.

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.

Ferlay J., Soerjomataram I., Ervik M., Dikshit R., Eser S., Mathers C., Rebelo M., Parkin D., Forman D. and Bray F. (2012) GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: IARC.

Fife K.H., Cramer H.M., Schroeder J.M. and Brown D.R. (2001) Detection of multiple human papillomavirus types in the lower genital tract correlates with cervical dysplasia***. *J Med Virol* 64, 550-559.

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.

Franceschi S., Herrero R., Clifford G.M., Snijders P.J., Arslan A., Anh P.T., Bosch F.X., Ferreccio C., Hieu N.T., Lazcano-Ponce E., Matos E., Molano M., Qiao Y.L., Rajkumar R., Ronco G., de Sanjose S., Shin H.R., Sukvirach S., Thomas J.O.,

Meijer C.J. and Munoz N. (2006) Variations in the age-specific curves of human papillomavirus prevalence in women worldwide. *International journal of cancer* 119, 2677-2684.

Franceschi S., Rajkumar T., Vaccarella S., Gajalakshmi V., Sharmila A., Snijders P.J., Munoz N., Meijer C.J. and Herrero R. (2003) Human papillomavirus and risk factors for cervical cancer in Chennai, India: a case-control study. *International journal of cancer* 107, 127-133.

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.

GAVI (2014) Ghana to vaccinate girls against HPV with GAVI support. Geneva: WHO.

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.

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.

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.

Gravitt P., Peyton C., Alessi T., Wheeler C., Coutlee F., Hildesheim A., Schiffman M., Scott D. and Apple R. (2000) Improved amplification of genital human papillomaviruses. *Journal of Clinical Microbiology* 38, 357-361.

Gravitt P., Peyton C., Apple R. and Wheeler C. (1998) Genotyping of 27 human papillomavirus types by using L1 consensus PCR products by a single-hybridization, reverse line blot detection method. *Journal of Clinical Microbiology* 36, 3020-3027.

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.

Halbert C.L., Demers G.W. and Galloway D.A. (1991) The E7 gene of human papillomavirus type 16 is sufficient for immortalization of human epithelial cells. *Journal of Virology* 65, 473-478.

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.

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.

Hawley-Nelson P., Vousden K.H., Hubbert N.L., Lowy D.R. and Schiller J.T. (1989) HPV16 E6 and E7 proteins cooperate to immortalize human foreskin keratinocytes. *The EMBO journal* 8, 3905-3910.

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.

Herrero R., Hildesheim A., Bratti C., Sherman M.E., Hutchinson M., Morales J., Balmaceda I., Greenberg M.D., Alfaro M., Burk R.D., Wacholder S., Plummer M. and Schiffman M. (2000) Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. *Journal of the National Cancer Institute* 92, 464-474.

Hibbitts S., Rieck G.C., Hart K., Powell N.G., Beukenholdt R., Dallimore N., McRea J., Hauke A., Tristram A. and Fiander A.N. (2006) Human papillomavirus infection: an anonymous prevalence study in South Wales, UK. *British Journal of Cancer* 95, 226-232.

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.

Horrigan E. and Herrington C. (2006) HPV and cervical cytology. *Current Diagnostic Pathology* 12, 98-103.

Husnjak K., Grce M., Magdic L. and Pavelic K. (2000) Comparison of five different polymerase chain reaction methods for detection of human papillomavirus in cervical cell specimens. *Journal of Virology Methods* 88, 125-134.

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

IARC (2004) Cervix Cancer Screening. In *Handbooks of Cancer Prevention*. Lyon: International Agency for Research on Cancer.

IARC (2007) Monograph on Papillomaviruses. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*. Lyon, France.

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

Jacobs M., Walboomers J., Snijders P., Voorhorst F., Daalmeijer N. and Meijer C. (2000) Age-related distribution patterns of 37 mucosotropic HPV types in women with cytologically normal cervical smears: decreased high risk/low risk ratio at older age. *International journal of cancer* 87, 221 - 227.

Jacobs M.V., de Roda Husman A.M., van den Brule A.J., Snijders P.J., Meijer C.J. and Walboomers J.M. (1995) Group-specific differentiation between high- and lowrisk human papillomavirus genotypes by general primer-mediated PCR and two cocktails of oligonucleotide probes. *Journal of Clinical Microbiology* 33, 901-905.

Jacobs M.V., Snijders P.J., van den Brule A.J., Helmerhorst T.J., Meijer C.J. and Walboomers J.M. (1997) A general primer GP5+/GP6(+)-mediated PCRenzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *Journal of Clinical Microbiology*
35, 791-795.

Jensen K., Schmiedel S., Itner T., Frederiksen K. and Kjær S. (2011) Parity as a CoFactor for Subsequent CIN3+ Among HPV-Positive Women. *27th International Papillomavirus Conference and Clinical Workshop*, 03:06.

Juskevicius R., Zou K.H. and Cibas E.S. (2001) An Analysis of Factors that Influence the ASCUS/SILRatio of Pathologists. *American journal of clinical pathology* 116, 331-335.

Kahn J.A., Rosenthal S.L., Succop P.A., Ho G.Y. and Burk R.D. (2002) The interval between menarche and age of first sexual intercourse as a risk factor for subsequent HPV infection in adolescent and young adult women. *J Pediatr* 141, 718-723.

Kashyap V., Hedau S. and Bhambhani S. (2011) Defining the validity of classical and non-classical cellular changes indicative of low-grade squamous intraepithelial lesion encompassing human papillomavirus infection in relation to human papillomavirus deoxyribonucleic acid testing. *J Cytol* 28, 159-164.

Kay P., Soeters R., Nevin J., Denny L., Dehaeck C. and Williamson A.L. (2003) High prevalence of HPV 16 in South African women with cancer of the cervix and cervical intraepithelial neoplasia. *J Med Virol* 71, 265-273.

Kelly D., Kincaid E., Fansler Z., Rosenthal D.L. and Clark D.P. (2006) Detection of cervical high-grade squamous intraepithelial lesions from cytologic samples using a novel immunocytochemical assay (ProEx C). *Cancer* 108, 494-500.

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) Highrisk 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., Bock J.E., Poll P.A., Engholm G., Sherman M.E., Walboomers J. and Meijer C. (1997) Determinants for genital human papillomavirus (HPV) infection in 1000 randomly chosen young Danish women with normal Pap smear: are there different risk profiles for oncogenic and nononcogenic HPV types? *Cancer Epidemiology Biomarkers & Prevention* 6, 799-805.

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.

Kjellberg L., Hallmans G., Ahren A.M., Johansson R., Bergman F., Wadell G., Angstrom T. and Dillner J. (2000) Smoking, diet, pregnancy and oral contraceptive use as risk factors for cervical intra-epithelial neoplasia in relation to human papillomavirus infection. *British Journal of Cancer* 82, 1332-1338.

Klaes R., Friedrich T., Spitkovsky D., Ridder R., Rudy W., Petry U., Dallenbach-Hellweg G., Schmidt D. and von Knebel Doeberitz M. (2001) Overexpression of p16(INK4A) as a specific marker for dysplastic and neoplastic epithelial cells of the cervix uteri. *International journal of cancer* 92, 276-284.

Kleter B., van Doorn L.-J., ter Schegget J., Schrauwen L., van Krimpen K., Burger M., ter Harmsel B. and Quint W. (1998a) Novel short-fragment PCR assay for highly

sensitive broad-spectrum detection of anogenital human papillomaviruses. *The American journal of pathology* 153, 1731-1739.

Kleter B., van Doorn L.J., ter Schegget J., Schrauwen L., van Krimpen K., Burger M., ter Harmsel B. and Quint W. (1998b) Novel short-fragment PCR assay for highly sensitive broad-spectrum detection of anogenital human papillomaviruses. *The Journal of pathology* 153, 1731-1739.

Klionsky D.J., Abeliovich H., Agostinis P., Agrawal D.K., Aliev G., Askew D.S., Baba M., Baehrecke E.H., Bahr B.A., Ballabio A., Bamber B.A., Bassham D.C., Bergamini E., Bi X., Biard-Piechaczyk M., Blum J.S., Bredesen D.E., Brodsky J.L., Brumell J.H., Brunk U.T., Bursch W., Camougrand N., Cebollero E., Cecconi F., Chen Y., Chin L.S., Choi A., Chu C.T., Chung J., Clarke P.G., Clark R.S., Clarke S.G., Clave C., Cleveland J.L., Codogno P., Colombo M.I., CotoMontes A., Cregg J.M., Cuervo A.M., Debnath J., Demarchi F., Dennis P.B., Dennis P.A., Deretic V., Devenish R.J., Di Sano F., Dice J.F., Difiglia M., Dinesh-Kumar S., Distelhorst C.W., Djavaheri-Mergny M., Dorsey F.C., Droge W., Dron M., Dunn W.A., Jr., Duszenko M., Eissa N.T., Elazar Z., Esclatine A., Eskelinen E.L., Fesus L., Finley K.D., Fuentes J.M., Fueyo J., Fujisaki K., Galliot B., Gao F.B., Gewirtz D.A., Gibson S.B., Gohla A., Goldberg A.L., Gonzalez R., Gonzalez-Estevez C., Gorski S., Gottlieb R.A., Haussinger D., He Y.W., Heidenreich K., Hill J.A., Hoyer-Hansen M., Hu X., Huang W.P., Iwasaki A., Jaattela M., Jackson W.T., Jiang X., Jin S., Johansen T., Jung J.U., Kadowaki M., Kang C., Kelekar A., Kessel D.H., Kiel J.A., Kim H.P., Kimchi A., Kinsella T.J., Kiselyov K., Kitamoto K., Knecht E., Komatsu M., Kominami E., Kondo S., Kovacs A.L., Kroemer G., Kuan C.Y., Kumar R., Kundu M., Landry J., Laporte M., Le W., Lei H.Y., Lenardo M.J., Levine B., Lieberman A., Lim K.L., Lin F.C., Liou W., Liu L.F., Lopez-Berestein G., Lopez-Otin C., Lu B., Macleod K.F., Malorni W., Martinet W., Matsuoka K., Mautner J., Meijer A.J., Melendez A., Michels P., Miotto G., Mistiaen W.P., Mizushima N., Mograbi B., Monastyrska I., Moore M.N., Moreira P.I., Moriyasu Y., Motyl T., Munz C., Murphy L.O., Naqvi N.I., Neufeld T.P., Nishino I., Nixon R.A., Noda T., Nurnberg B., Ogawa M., Oleinick N.L., Olsen L.J., Ozpolat B., Paglin S., Palmer G.E., Papassideri I., Parkes M., Perlmutter D.H., Perry G., Piacentini M., Pinkas-Kramarski R., Prescott M., Proikas-Cezanne T., Raben N., Rami A., Reggiori F., Rohrer B., Rubinsztein D.C., Ryan K.M., Sadoshima J., Sakagami H., Sakai Y., Sandri M., Sasakawa C., Sass M., Schneider C., Seglen P.O., Seleverstov O., Settleman J., Shacka J.J., Shapiro I.M., Sibirny A., Silva-Zacarin E.C., Simon H.U., Simone C., Simonsen A., Smith M.A., Spanel-Borowski K., Srinivas V., Steeves M., Stenmark H., Stromhaug P.E., Subauste C.S., Sugimoto S., Sulzer D., Suzuki T., Swanson M.S., Tabas I., Takeshita F., Talbot N.J., Talloczy Z., Tanaka K., Tanida I., Taylor G.S., Taylor J.P., Terman A., Tettamanti G., Thompson C.B., Thumm M., Tolkovsky A.M., Tooze S.A., Truant R., Tumanovska L.V., Uchiyama Y., Ueno T., Uzcategui N.L., van der Klei I., Vaquero E.C., Vellai T., Vogel M.W., Wang H.G., Webster P., Wiley J.W., Xi Z., Xiao G., Yahalom J., Yang J.M., Yap G., Yin X.M., Yoshimori T., Yu L., Yue Z., Yuzaki M., Zabirnyk O., Zheng X., Zhu X. and Deter R.L. (2008) Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. *Autophagy* 4, 151-175.

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. and Melamed M. (2005) Koss' Squamous Carcinoma of the Uterine Cervix and its Precursors. In *Diagnostic Cytology and its Histopathologic Basis*, pp. 287. Philadelphia: Lippincott Williams and Wilkins.

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

Koutsky L.A. and Kiviat N.B. (1999) *Genital Human Papillimavirus*: New York: McGraw Hill.

Kraus I., Molden T., Erno L.E., Skomedal H., Karlsen F. and Hagmar B. (2004) Human papillomavirus oncogenic expression in the dysplastic portio; an investigation of biopsies from 190 cervical cones. *British Journal of Cancer* 90, 1407-1413.

Kraus I., Molden T., Holm R., Lie A.K., Karlsen F., Kristensen G.B. and Skomedal H. (2006) Presence of E6 and E7 mRNA from human papillomavirus types 16, 18, 31, 33, and 45 in the majority of cervical carcinomas. *Journal of Clinical Microbiology* 44, 1310-1317.

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.

Lavia P., Mileo A.M., Giordano A. and Paggi M.G. (2003) Emerging roles of DNA tumor viruses in cell proliferation: new insights into genomic instability. *Oncogene* 22, 6508-6516.

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.

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., Hildesheim A., Burk R.D., Gravitt P., Wacholder S., Manos M.M., Scott D.R., Sherman M.E., Kurman R.J. and Glass A.G. (2001a) 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.

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. (2001b) 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.

Liaw K.L., Hsing A.W., Schiffman M.H., You S.L., Zhang T., Burk R. and Chen C.J. (1997) Human papillomavirus types 52 and 58 are prevalent in cervical cancer from Chinese women. *International journal of cancer* 73, 775-776.

Lie A.K. and Kristensen G. (2008) Human papillomavirus E6/E7 mRNA testing as a predictive marker for cervical carcinoma. *Expert Rev Mol Diagn* 8, 405-415.

Longworth M.S. and Laimins L.A. (2004) Pathogenesis of human papillomaviruses in differentiating epithelia. *Microbiol Mol Biol Rev* 68, 362-372.

Lorincz A.T., Reid R., Jenson A.B., Greenberg M.D., Lancaster W. and Kurman R.J. (1992) Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obstetrics and Gynecology* 79, 328-337.

Lowy D.R. and Howley P.M. (2001) Fields virology. In *Papillomaviruses.* , pp. 2231–2264 [D.M. Knipe and P.M. Howley, editors]. Philadelphia, USA: Lippincott, Williams and Wilkins.

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.

Luque A.E., Demeter L.M. and Reichman R.C. (1999) Association of human papillomavirus infection and disease with magnitude of human immunodeficiency virus type 1 (HIV-1) RNA plasma level among women with HIV-1 infection. *Journal of Infectious Diseases* 179, 1405-1409.

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.

Manos M., Ting Y., Wright D., Lewis A., Broker T. and Wolinsky S. (1989a) Use of polymerase chain reaction amplification for the detection of genital human papillomavirus. *Cancer Cells* 7, 209 - 214.

Manos M., Ting Y., Wright D., Lewis A., Broker T. and Wolinsky S. (1989b) Use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* 7, 209-214.

Marie T.P., Sando Z., Ndoumba A., Sandjong I., Mawech-Fauceglia P. and Doh A.S. (2013) Prevalence and Geographical Distribution of Precancerous Lesions of the Uterine Cervix in Cameroon. *Journal of Cytology & Histology* 2013.

Masciullo V., Khalili K. and Giordano A. (2000) The Rb family of cell cycle regulatory factors: clinical implications. *International Journal of Oncology* 17, 897-902.

Matos E., Loria D., Amestoy G.M., Herrera L., Prince M.A., Moreno J., Krunfly C., van den Brule A.J., Meijer C.J., Munoz N. and Herrero R. (2003) Prevalence of human papillomavirus infection among women in Concordia, Argentina: a population-based study. *Sexually Transmitted Diseases* 30, 593-599.

Matzow T., Boysen M., Kalantari M., Johansson B. and Hagmar B. (1998) Low detection rate of HPV in oral and laryngeal carcinomas. *Acta Oncologica* 37, 73-76.

Mayaud P., Weiss H.A., Lacey C.J., Gill D.K. and Mabey D.C. (2003) Genital human papillomavirus genotypes in northwestern Tanzania. *Journal of Clinical Microbiology* 41, 4451-4453.

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.

Méndez F., Muñoz N., Posso H., Molano M., Moreno V., van den Brule A.J., Ronderos M., Meijer C. and Group I.N.d.C.H.P.S. (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.

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.

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

Mitchell V. (1996) Assessing the reliability and validity of questionnaires: an empirical example. *Journal of Applied Management Studies* 5, 199-208.

MOH (2014) Ghana Immunization Programme Comprehensive Multiyear Plan (20102014). Accra, Ghana: Ministry of Health.

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.

Molden T., Kraus I., Skomedal H., Nordstrom T. and Karlsen F. (2007) PreTect HPVProofer: real-time detection and typing of E6/E7 mRNA from carcinogenic human papillomaviruses. *Journal of Virology Methods* 142, 204-212.

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, 3745.

Motoyama S., Ladines-Llave C.A., Luis Villanueva S. and Maruo T. (2004) The role of human papilloma virus in the molecular biology of cervical carcinogenesis. *Kobe J Med Sci* 50, 9-19.

Munger K., Baldwin A., Edwards K.M., Hayakawa H., Nguyen C.L., Owens M., Grace M. and Huh K. (2004) Mechanisms of human papillomavirus-induced oncogenesis. *Journal of Virology* 78, 11451-11460.

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

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

Munger K., Phelps W.C., Bubb V., Howley P.M. and Schlegel R. (1989) The E6 and E7 genes of the human papillomavirus type 16 together are necessary and sufficient for transformation of primary human keratinocytes. *Journal of Virology* 63, 4417-4421.

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. (2004a) 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.

Munoz N., Bosch F.X., deSanjose S., Vergara A., delMora A., Munoz M.T., Tafur L., Gili M., izarzugaza I., Viladiu P., Navarro C., Alonso de Ruiz P., Aristizabal N., Santamaria M., Orfila J., Daniel R.W., Guerrero E. and Shah K.V. (1993) Risk factors

for cervical intraepithelial neoplasia grade III/carcinoma in situ in Spain and Colombia. *Cancer Epidemiology Biomarkers & Prevention* 2, 423-431.

Munoz N., Castellsague X., de Gonzalez A.B. and Gissmann L. (2006) Chapter 1: HPV in the etiology of human cancer. *Vaccine* 24 Suppl 3, S3/1-10.

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.

Munoz N., Mendez F., Posso H., Molano M., van den Brule A.J., Ronderos M., Meijer C. and Munoz A. (2004b) Incidence, duration, and determinants of cervical human papillomavirus infection in a cohort of Colombian women with normal cytological results. *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.

Nascimento A.F. and Cibas E.S. (2007) The ASC/SIL ratio for cytopathologists as a quality control measure. *American journal of clinical pathology* 128, 653-656.

Naucler P., Ryd W., Tornberg S., Strand A., Wadell G., Elfgren K., Radberg T., Strander B., Johansson B., Forslund O., Hansson B.G., Rylander E. and Dillner J. (2007) Human papillomavirus and Papanicolaou tests to screen for cervical cancer. *New England Journal of Medicine* 357, 1589-1597.

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.

Novelli G., Gennarelli M., De Santis L., Angeloni P. and Dallapiccola B. (1992) Inosinecontaining primers in human papillomavirus detection by polymerase chain reaction. *Biomed Pharmacother* 46, 167-169.

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

Parkin D., Whelan S., Ferlay J., Teppo L. and Thomas D. (2002) Cancer incidence in five continents Vol. VIII. *IARC scientific publications* 155.

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.

Pehr J.L. and Akuamoa-Boateng A. (2010) Health Needs Assessment for Kumasi, Ghana. In *MCI Social Sector Working Paper Series*.

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., Schmidt D., Scherbring S., Luyten A., Reinecke-Luthge A., Bergeron C., Kommoss F., Loning T., Ordi J., Regauer S. and Ridder R. (2011) Triaging Pap cytology negative, HPV positive cervical cancer screening results with p16/Ki67 Dual-stained cytology. *Gynecol Oncol* 121, 505-509.

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.

Pfister H. (2003) Human papillomavirus and skin cancer. *Journal of the National Cancer Institute Monographs* 31, 52-56.

Pim D., Bergant M., Boon S.S., Ganti K., Kranjec C., Massimi P., Subbaiah V.K., Thomas M., Tomaić V. and Banks L. (2012) Human papillomaviruses and the specificity of PDZ domain targeting. *FEBS Journal* 279, 3530-3537.

Piras F., Piga M., De Montis A., Zannou A.R., Minerba L., Perra M.T., Murtas D., Atzori M., Pittau M. and Maxia C. (2011) Prevalence of human papillomavirus infection in women in Benin, West Africa. *Virology journal* 8, 1.

Pirisi L., Yasumoto S., Feller M., Doniger J. and DiPaolo J.A. (1987) Transformation of human fibroblasts and keratinocytes with human papillomavirus type 16 DNA. *Journal of Virology*
61, 1061-1066.

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.

Poljak M., Marin I.J., Seme K. and Vince A. (2002) Hybrid Capture II HPV Test detects at least 15 human papillomavirus genotypes not included in its current high-risk probe cocktail. *Journal of clinical virology* 25 Suppl 3, S89-97.

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.

Ratnam S., Coutlee F., Fontaine D., Bentley J., Escott N., Ghatage P., Gadag V., Holloway G., Bartellas E., Kum N., Giede C. and Lear A. (2011) Aptima HPV E6/E7 mRNA test is as sensitive as Hybrid Capture 2 Assay but more specific at detecting cervical precancer and cancer. *Journal of Clinical Microbiology* 49, 557-564.

Resnick R.M., Cornelissen M.T., Wright D.K., Eichinger G.H., Fox H.S., ter Schegget J. and Manos M.M. (1990) Detection and typing of human papillomavirus in archival cervical cancer specimens by DNA amplification with consensus primers. *Journal of the National Cancer Institute* 82, 1477-1484.

Robboy S.J., Merino M.J. and Mutter G.L. (2008) *Pathogenesis of HPV infection*, 5 ed: Lippincott Williams & Wilkins.

Rolón P.A., Smith J.S., Muñoz N., Klug S.J., Herrero R., Bosch X., Llamosas F., Meijer C.J. and Walboomers J.M. (2000) Human papillomavirus infection and invasive cervical cancer in Paraguay. *International journal of cancer* 85, 486-491.

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

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

Safaeian M., Herrero R., Hildesheim A., Quint W., Freer E., Van Doorn L.J., Porras C., Silva S., Gonzalez P., Bratti M.C., Rodriguez A.C. and Castle P. (2007) Comparison of the SPF10-LiPA system to the Hybrid Capture 2 Assay for detection of carcinogenic human papillomavirus genotypes among 5,683 young women in Guanacaste, Costa Rica. *Journal of Clinical Microbiology* 45, 1447-1454.

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.

Said H.M., Ahmed K., Burnett R., Allan B., Williamson A.-L. and Hoosen A.A. (2009) HPV genotypes in women with squamous intraepithelial lesions and normal cervixes participating in a community-based microbicide study in Pretoria, South Africa. *Journal of clinical virology* 44, 318-321.

Sasagawa T., Basha W., Yamazaki H. and Inoue M. (2001) High-risk and multiple human papillomavirus infections associated with cervical abnormalities in Japanese women. *Cancer Epidemiology Biomarkers & Prevention* 10, 45-52.

Sasagawa T., Minemoto Y., Basha W., Yamazaki H., Nakamura M., Yoshimoto H., Sakaike J. and Inoue M. (2000) A new PCR-based assay amplifies the E6–E7 genes of most mucosal human papillomaviruses (HPV). *Virus research* 67, 127139.

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.

Sawaya G.F., Brown A.D., Washington A.E. and Garber A.M. (2001) Current approaches to cervical-cancer screening. *New England Journal of Medicine* 344, 1603-1607.

Schacter J., Hill E.C., King E.B., Heilbron D.C., Ray R.M., Margolis A.J. and Greenwood S.A. (1982) Chlamydia trachomatis and cervical metaplasia. *J Amer Med Ass.* 248, 2134-2138.

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., DuarteFranco 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.

Schmitt M., Depuydt C., Benoy I., Bogers J., Antoine J., Arbyn M. and Pawlita M. (2013a) Prevalence and viral load of 51 genital human papillomavirus types and three subtypes. *International journal of cancer* 132, 2395-2403.

Schmitt M., Depuydt C., Benoy I., Bogers J., Antoine J., Arbyn M., Pawlita M. and Group V.S. (2013b) Prevalence and viral load of 51 genital human papillomavirus types and three subtypes. *International journal of cancer* 132, 23952403.

Schwarz E., Freese U.K., Gissmann L., Mayer W., Roggenbuck B., Stremlau A. and zur Hausen H. (1985) Structure and transcription of human papillomavirus sequences in cervical carcinoma cells. *Nature* 314, 111-114.

Sellors J.W., Mahony J.B., Kaczorowski J., Lytwyn A., Bangura H., Chong S., Lorincz A., Dalby D.M., Janjusevic V. and Keller J.L. (2000) Prevalence and predictors of human papillomavirus infection in women in Ontario, Canada. Survey of HPV in Ontario Women (SHOW) Group. *CMAJ* 163, 503-508.

Serwadda D., Wawer M.J., Shah K.V., Sewankambo N.K., Daniel R., Li C., Lorincz A., Meehan M.P., Wabwire-Mangen F. and Gray R.H. (1999) Use of a hybrid capture assay of self-collected vaginal swabs in rural Uganda for detection of human papillomavirus. *Journal of Infectious Diseases* 180, 1316-1319.

Smith R.A., Cokkinides V., von Eschenbach A.C., Levin B., Cohen C., Runowicz C.D., Sener S., Saslow D. and Eyre H.J. (2002) American Cancer Society guidelines for the early detection of cancer. *CA: a cancer journal for clinicians* 52, 8-22.

Smits H.L., Tieben L.M., Tjong A.H.S.P., Jebbink M.F., Minnaar R.P., Jansen C.L. and ter Schegget J. (1992) Detection and typing of human papillomaviruses present in fixed and stained archival cervical smears by a consensus polymerase chain reaction and direct sequence analysis allow the identification of a broad spectrum of human papillomavirus types. *J gen Virol* 73 (Pt 12), 3263-3268.

Snijders P.J., van den Brule A.J., Schrijnemakers H.F., Snow G., Meijer C.J. and Walboomers J.M. (1990) The use of general primers in the polymerase chain reaction permits the detection of a broad spectrum of human papillomavirus genotypes. *J gen Virol* 71 (Pt 1), 173-181.

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.

Solomon D., Schiffman M. and Tarone R. (2001) Comparison of three management strategies for patients with atypical squamous cells of undetermined significance: baseline results from a randomized trial. *Journal of the National Cancer Institute* 93, 293-299.

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.

Soudre B., Lamien A., Kone B., Sanou M. and Sakande B. (1992) Les lésions précancereuses et cancéreuses du col utérin au Burkina-Faso: étude cytologique réalisée dans deux provinces (Oudalan, Séno). *Médecine d'Afrique noire* 39, 806809.

Stamataki P., Papazafiropoulou A., Elefsiniotis I., Giannakopoulou M., Brokalaki H., Apostolopoulou E., Sarafis P. and Saroglou G. (2010) Prevalence of HPV infection among Greek women attending a gynecological outpatient clinic. *BMC infectious diseases* 10, 27.

Stanley M., Lowy D.R. and Frazer I. (2006) Prophylactic HPV vaccines: underlying mechanisms. *Vaccine* 24, S106-S113.

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.

Syrajnen K.J., Mantyjarvi R., Vayrynen M., Yliskoski M., Syrajnen S.M. and Saarikoski S. (1987) Cervical smears in assessment of the natural history of cervical human

papillomavirus infections in prospectively followed women. *Acta Cytol* 31, 855–865.

Szarewski A., Ambroisine L., Cadman L., Austin J., Ho L., Terry G., Liddle S., Dina R., McCarthy J., Buckley H., Bergeron C., Soutter P., Lyons D. and Cuzick J. (2008) Comparison of predictors for high-grade cervical intraepithelial neoplasia in women with abnormal smears. *Cancer Epidemiology Biomarkers & Prevention* 17, 3033-3042.

Temmerman M., Tyndall M.W., Kidula N., Claeys P., Muchiri L. and Quint W. (1999) Risk factors for human papillomavirus and cervical precancerous lesions, and the role of concurrent HIV-1 infection. *International Journal Gynaecology and Obstetrics* 65, 171-181.

Thomas J.O., Herrero R., Omigbodun A.A., Ojemakinde K., Ajayi I.O., Fawole A., Oladepo O., Smith J.S., Arslan A., Munoz N., Snijders P.J.F., Meijer C.J.L.M. and Franceschi S. (2004) Prevalence of papillomavirus infection in women in Ibadan, Nigeria: a population based study. *British Journal of Cancer* 90, 638-645.

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

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

Thomas M. and Banks L. (1998) Inhibition of Bak-induced apoptosis by HPV-18 E6. *Oncogene* 17, 2943-2954.

Thomas M., Pim D. and Banks L. (1999) The role of the E6-p53 interaction in the molecular pathogenesis of HPV. *Oncogene* 18, 7690-7700.

Trottier H. and Franco E.L. (2006) The epidemiology of genital human papillomavirus infection. *Vaccine* 24 Suppl 1, S1-15.

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. (2006) 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.

Vaccarella S., Franceschi S., Snijders P.J., Herrero R., Meijer C.J., Plummer M. and Group I.H.P.S.S. (2010) Concurrent infection with multiple human papillomavirus types: pooled analysis of the IARC HPV Prevalence Surveys. *Cancer Epidemiology Biomarkers & Prevention* 19, 503-510.

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., Meijer C.J., Bakels V., Kenemans P. and Walboomers J.M. (1990) Rapid detection of human papillomavirus in cervical scrapes by combined general primer-mediated and type-specific polymerase chain reaction. *Journal of Clinical Microbiology* 28, 2739-2743.

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.

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.

von Knebel-Doeberitz M. and Syrjanen K.J. (2006) Molecular markers: how to apply in practice. *Gynecol Oncol* 103, 18-20.

von Knebel Doeberitz M., Rittmuller C., zur Hausen H. and Durst M. (1992) Inhibition of tumorigenicity of cervical cancer cells in nude mice by HPV E6-E7 anti-sense RNA. *International journal of cancer* 51, 831-834.

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.

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.

Werness B.A., Levine A.J. and Howley P.M. (1990) Association of human papillomavirus types 16 and 18 E6 proteins with p53. *Science* 248, 76-79.

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

WHO (2012) Prevention of cervical cancer through screening using visual inspection with acetic acid (VIA) and treatment with cryotherapy. A demonstration project in six African countries: Malawi, Madagascar, Nigeria, Uganda, the United Republic of Tanzania, and Zambia [WHO, editor: WHO libr Cat Data.

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. (2000a) Paratesticular liposarcoma with smooth muscle differentiation mimicking angiomyolipoma. *Histopathology* 36, 221-223.

Womack S.D., Chirenje Z.M., Blumenthal P.D., Gaffikin L., McGrath J.A., Chipato T., Ngwalle E. and Shah K.V. (2000b) Evaluation of a human papillomavirus assay in cervical screening in Zimbabwe. *BJOG* 107, 33-38.

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, 18311836.

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., Jr., Massad L.S., Dunton C.J., Spitzer M., Wilkinson E.J. and Solomon D. (2007a) 2006 consensus guidelines for the management of women with abnormal cervical screening tests. *J Low Genit Tract Dis* 11, 201-222.

Wright T.C., Jr., Massad L.S., Dunton C.J., Spitzer M., Wilkinson E.J. and Solomon D. (2007b) 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ. *J Low Genit Tract Dis* 11, 223239.

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.

Wright T.C., Stoler M.H., Behrens C.M., Sharma A., Zhang G. and Wright T.L. (2015) Primary cervical cancer screening with human papillomavirus: End of study results from the ATHENA study using HPV as the first-line screening test. *Gynecol Oncol*.

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, WestAfrican women over 35 years of age. *International journal of cancer* 103, 803-809.

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*.

Yoshikawa H., Kawana T., Kitagawa K., Mizuno M., Yoshikura H. and Iwamoto A. (1990) Amplification and typing of multiple cervical cancer-associated human papillomavirus DNAs using a single pair of primers. *International journal of cancer* 45, 990-992.

Yoshikawa H., Kawana T., Kitagawa K., Mizuno M., Yoshikura H. and Iwamoto A. (1991) Detection and typing of multiple genital human papillomaviruses by DNA amplification with consensus primers. *Japanese Journal of Cancer Research* 82, 524-531.

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.

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

Zur Hausen H. (1996b) Papillomavirus infections—a major cause of human cancers. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer* 1288, F55-F78.

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

APPENDIX 1

PATIENT QUESTIONNAIRE

Appendix 1: Patient Questionnaire

Identification Information

I 1	Date: **____ /____ / 20____** dd-mm-yyyy	*LMP: **____ /____ / 20____** dd-mm-yyyy	time:	interviewer:

					Data Entry
I 2	**Consent**	Consent has been read out to respondent and obtained (written)	Yes 1 No 2	**If NO, END**	
I 3	**Exclusion criteria**	Participant is eligible to participate in the study.	Yes 1 No 2	**If NO, END**	

I 4	Family name:	Other names:
I 5	Contact phone number(s):	House address:

*LMP: last menstrual period

Please circle the appropriate answer or indicate response in the space provided

Demographic Information

				Data Entry
D1	**AGE**	How old are you?	.....years Don't know 77 Refused 88	
D2	**Menarche:**	**At what age did you have your first menstrual period?**	.....years Don't know 77 Refused 88	

D3	**Menopause:**	**If post-menopausal, at what age did you have your last menstrual period?**	.....years Don't know 77 Refused 88	

D4	**Coitache:**	**At what age did you have your first sexual experience?**	.....years Don't know 77 Refused 88	

Par	**Parity:** Please record parity as follows:

Gravida	**Para**	**Abortion**	**Induced**	**Spontaneous**

D5	**Age at first pregnancy**	How old were you when you had your first pregnancy?	..... Don't know 77 Refused 88	
D6		What is your ethnic group? *When in doubt simply indicate hometown.*	Akan 1 Mole-Dagbane 2 Ewe 3 Ga-Adangbe 4 other (specify) 5	
D7		What is the highest level of education you have completed?	Never attended school 1 Primary school 2 Middle school 3 Junior Sec. school 4 Sec./ high school (6th form) 5 Technical training or equivalent 6 University or tertiary qualification 7 Don't know 77 Refused 88	

D8	Which of the following best describes your main work status over the last 12 months?	Government employee 1 Non-gov. Employee 2 Self-employed 3 National service 4 Subsistence farming 5 Trading 6 Student 7 Homemaker(household chores) Retired 8 Unemployed (able to work) 9 Unemployed (unable to work) 10 Don't know 77 Refused 88	
D9	What is your marital status?	Single 1 Married 2 Cohabiting 3 Separated/Divorced 4 Widowed 5 Don't know 77 Refused 88	
D10	What is your partner's age?	……years Don't know 77 Refused 88	

RISK FACTORS

Now we are going to ask some questions about various health behaviours. These include: sexual and reproductive history, contraception, smoking and alcohol intake.

Sexual and Reproductive History:

Sexual Partners

		Response			Data Entry
sexP1	In total, how many sexual partners have you had in your lifetime?	…… Don't know 77 Refused 88			
sexP2	Are you the only partner of your spouse?	Yes 1 No 2 Don't know 77 Refused 88		If **Yes**, go to **sexP6**	
sexP3	If **'No,'** how many partners in total does your spouse have presently?	…… Don't know 77 Refused 88			
sexP4	To your knowledge, was your current partner/spouse ever married to another woman before becoming your partner/husband?	Yes 1 No 2 Don't know 77 Refused 88			

sexP5	Does your current partner/spouse have other unmarried partners currently? If '**Yes**', please give number.	Yes No Don't know Refused	1 2 77 88	.....	
Contraception					
		Response			**Data Entry**
Con1	Have you ever used any contraceptives?	Yes No Don't know Refused	1 2 77 88	If **No**, go to **Con3**	
Con2	If 'Yes,' please indicate which contraceptives you used or are currently using.	N/A pill other both pill and other	0 1 2 3		35.1
Con3	Do you or your partner(s) have a habit of condom use?	Yes No Don't know Refused	1 2 77 88	35.2	35.3
Tobacco Use					
		Response		**Skip**	**Data Entry**
Tob	Do you currently smoke any **tobacco products,** such as cigarettes, cigars or pipes?	Yes No Don't know Refused	1 2 77 88	If **No**, go to **Tob3**	
Alcohol Consumption					
		Response			**Data Entry**
Alc1	Have you **ever consumed** a drink that contains alcohol such as beer, wine, spirit, bitters, palm wine or akpeteshie?	Yes No Don't know Refused	1 2 7 8	If **No**, go to Next Section	
Alc2	If 'Yes,' do you consume these drinks regularly (1 per month in the last 6 months)?	Yes No Don't know Refused	1 2 77 88		
Skin lightening					
Ble1	Have you **ever used** a skin product that is intended to make your skin fairer?	Yes No Don't know Refused	1 2 77 88	If **No**, go to Next Section	
Ble2	If 'Yes,' please indicate which product you used or are currently using.	..... Don't know 77 Refused 88			
Vaginal Washing					
Dou1	Do you have a habit of washing your private part?	Yes No Don't know Refused	1 2 77 88	If **No**, go to END	

Dou2	If 'Yes,' how often do you wash your vagina?	Rarely daily weekly Don't know Refused	1 2 3 77 88		

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

APPENDIX 3

MASTER MIX TABLE FOR GENERAL PRIMER PCR

Appendix 3 Master mix table for general primer PCR

REAGENTS	x1
10x PCR Buffer	3µl
20mM $MgCl_2$	1.5µl
d ATP	0.5µl
d GTP	0.5µl
d CTP	0.5µl
d TTP	0.5µl
[Primers]	
GP-E6-3F (10µl)	0.375µl
GP-E6-5B (10µl)	0.375µl
GP-E6-6B (10µl)	0.375µl
Template DNA	5µl
Nuclease-free water	appropriately
Taq Polymerase (5U/µl)	0.125µl
TOTAL:	25µl

APPENDIX 4

MASTER MIX TABLE FOR NESTED MULTIPLEX PCR

Appendix 4 Master mix table for Nested multiplex PCR

	Cocktail 1	Cocktail 2	Cocktail 3	Cocktail 4
REAGENTS	**x1**	**x1**	**x1**	**x1**
10x PCR Buffer	3µl	3µl	3µl	3µl
50mM $MgCl_2$	1.5µl	1.5µl	1.5µl	1.5µl
d ATP [10mM]	0.5µl	0.5µl	0.5µl	0.5µl
d GTP [10mM]	0.5µl	0.5µl	0.5µl	0.5µl
d CTP [10mM]	0.5µl	0.5µl	0.5µl	0.5µl
d TTP [10mM]	0.5µl	0.5µl	0.5µl	0.5µl
[Cocktail-specific primers] [10µM each]	0.375µl	0.375µl	0.375µl	0.375µl
Template DNA	2µl	2µl	2µl	2µl
Nuclease-free water	Appropriately	appropriately	appropriately	appropriately

Taq Polymerase (5U/µl)	0.125µl	0.125µl	0.125µl	0.125µl
TOTAL:	25µl	25µl	25µl	25µl

APPENDIX 5

Appendix 5: Combinations of human papillomavirus (HPV) types in 85 women with multiple infections

Double		Triple	Four	Five
HPV cluster	frequency	HPV cluster	HPV cluster	HPV cluster
16, 31		16, 42, 52 *	16, 39, 56, 58 *	16, 18, 42,43, 66 *
18, 42		16, 6/11, 42	35, 42, 39, 51	18, 45, 56, 42, 39
18, 43	2	18, 35, 56	42, 45, 52, 6/11 *	42, 44, 39, 66, 51
18, 52 *		18, 42, 56	45,35,43,68	
18, 68 *		18, 43, 52	52, 56, 35, 43	
35, 42 *	2	18, 45, 52*	52, 56, 58, 68 *	
35, 52 *	5	18, 52, 68	52, 58, 6/11, 68	
39, 42		18, 56, 68	52, 6/11, 39, 51	
39, 66		33, 39, 66	56, 43, 44, 68	
42, 43		33, 45, 52*		
42, 44		35, 52, 56		
42, 45		39, 56, 58		
42, 52	2	42, 52, 56 *		
43, 51	2	42, 52, 58		
43, 52		42, 52, 59		
43, 59		42, 52, 66		
43, 66		43, 51, 66		
45, 52		43, 56, 66		
52, 35		43, 56, 66		
52, 39		43, 56, 66		
52, 56	5	45, 33, 58		
52, 58		52, 58, 42		
52, 6/11		52, 58, 66		
52, 66 *	2	6/11, 42, 52		
52, 68		6/11, 43, 51		

56, 43		6/11, 43, 51
56, 58	4	
58, 68	2	

*Associated with abnormal cervical finding.

APPENDIX 6

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.

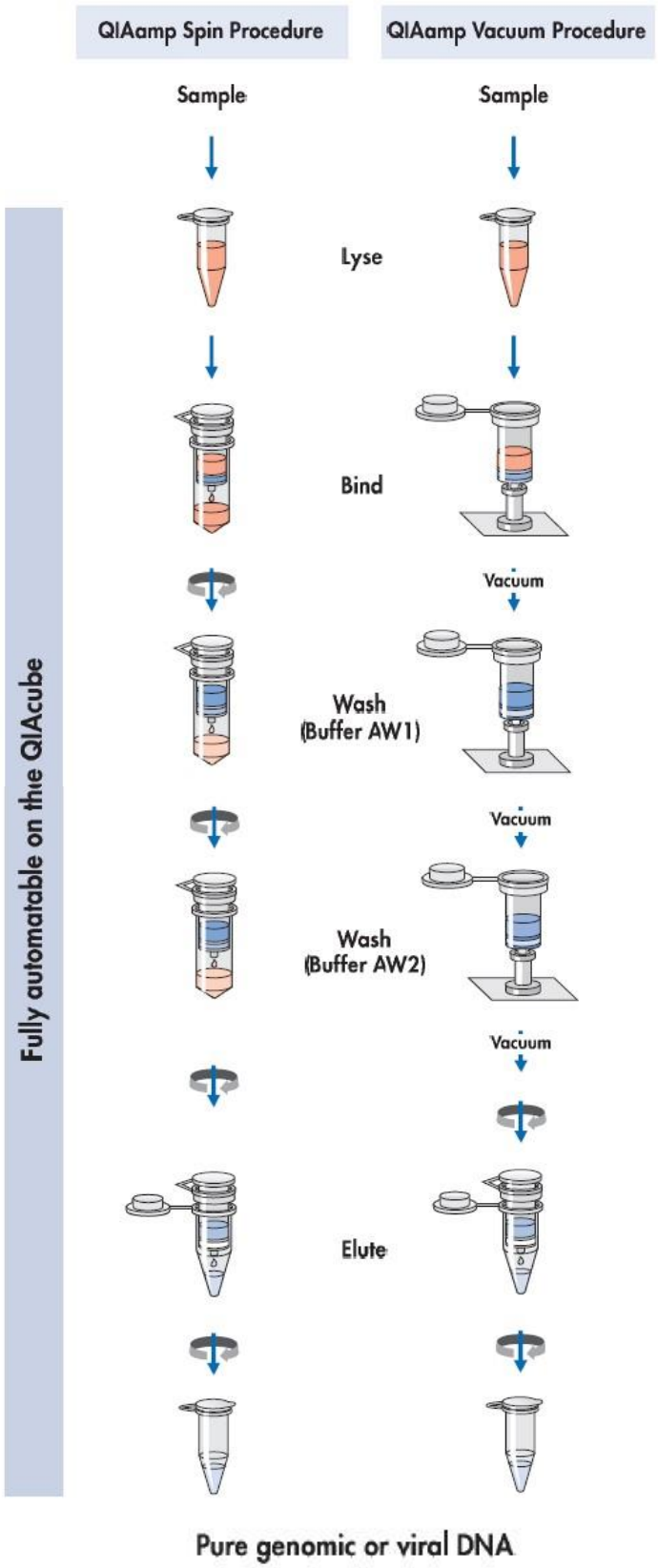
10. Place the QIAamp Mini spin column in a clean 1.5 ml microcentrifuge tube, and discard the collection tube containing the filtrate.

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°C.

Precautions:

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



Appendix 6: DNA Purification from Cervical Swabs using Qiagen DNA Mini kit (Spin Method). Adapted from QIAamp DNA Mini and Blood Mini Handbook 04/2010

APPENDIX 7

Formula without Finite Population Correction:

$$n = \frac{Z^2 P(1-P)}{d^2}$$

where n = Sample size,
Z = Z statistic for a level of confidence,
P = Expected prevalence or proportion
(If the expected prevalence is 20%, then $P = 0.2$), and
d = Precision (If the precision is 5%, then $d = 0.05$).

Formula with Finite Population Correction:

$$n' = \frac{NZ^2 P(1-P)}{d^2(N-1) + Z^2 P(1-P)}$$

where n' = Sample size with finite population correction,
N = Population size,
Z = Z statistic for a level of confidence,
P = Expected proportion (If the prevalence is 20%, $P = 0.2$), and
d = Precision (If the precision is 5%, then $d = 0.05$)

Appendix 7: Daniel WW (1999). Biostatistics: A Foundation for Analysis in the Health Sciences. 7th edn. New York: John Wiley & Sons