

The Prevalences of Human Papillomavirus Infections and Cervical Lesions, and
the Patterns of Risk Behaviour Characteristics of Women in a High Risk
Community: Akuse sub-district, Ghana

A Thesis Submitted by

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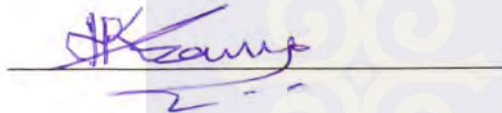
DECLARATION

I hereby declare that the work described in this thesis was performed by me, Adolf Kofi Awua, under the supervision of Professor R.M. Adanu - Dean of the School of Public Health, College of Health Sciences, University of Ghana, Legon; Professor E. K. Wiredu - Pro-Vice Chancellor, University of Health and Allied Sciences, Ho and Professor Col. (Rtd) E. A Afari - Department of Epidemiology and Disease Control, School of Public Health, University of Ghana, Legon. To the best of my knowledge, this thesis has not been submitted for any academic degree in any institution.

All references cited have been duly acknowledged.

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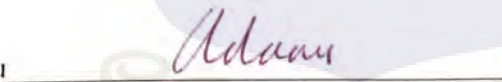
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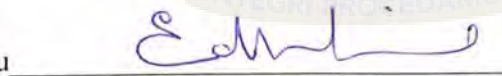
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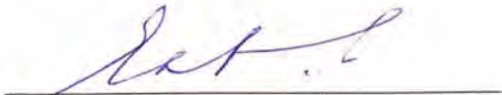
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DEDICATION

TO GOD AND MY FAMILY



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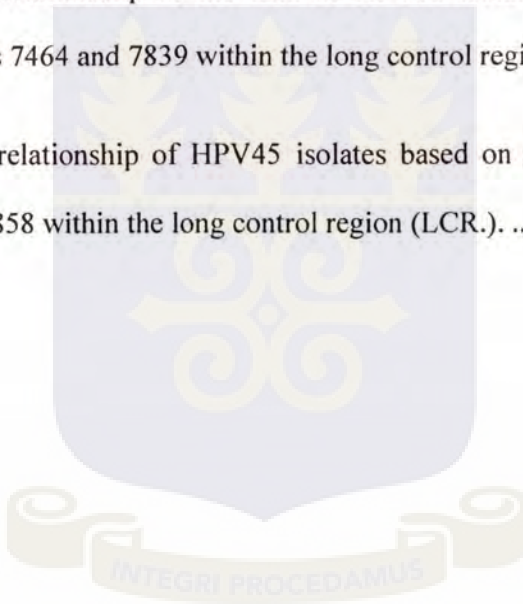
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LIST OF ABBREVIATIONS

ADC	Adenocarcinoma
ADSC	Adenosquamous Carcinoma
AFSI	Age at First Sexual Intercourse
AGC-US	Atypical Glandular Cells of Undetermined Significance
ASC-US	Atypical Squamous Cells of Undetermined Significance
ASR	Age-Standardized Rate
CIN	Cervical Intraepithelial Neoplasia
CIS	<i>Carcinoma-In Situ</i>
CNSP	Current Number of Male Partners
HPC	Health Personnel Collection
HPV	Human Papillomavirus
HR	High Risk
IARC	International Agency For Research on Cancer
IQR	Interquartile range
LBC	Liquid Based Cytology
LCR	Long Control Region
LNSP	Lifetime Number of Male Sexual Partners
LR	Low Risk

OC	Oral Contraceptive
OR	Odds Ratio
ORF	Open Reading Frames
PHR	Possibly High Risk
PPS	Probability Proportional to Size
SC	Self-Collection or Self-Collected
SCC	Squamous Cells Carcinoma
SCJ	Squamocolumnar Junction
SIL	Squamous Intraepithelial Lesion
SIR	Standardized Incidence Ratio
TZ	Transformational Zone
VIA	Visual Inspection with Acetic Acid
VILI	Visual Inspection with Lugol's Iodine
VLP	Viral-Like-Proteins



ABSTRACT

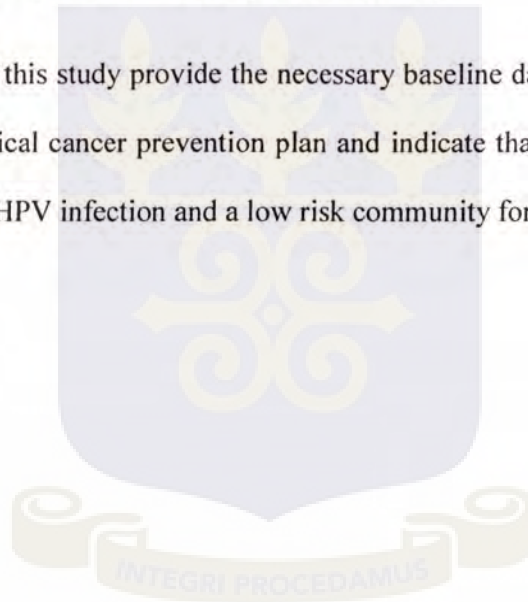
Background: Ghana continues to lack population based empirical data on the burden of cervical pre-cancer lesions, HPV infection and the identification of communities at risk. These together are hampering the control of cervical cancer in Ghana. Therefore, this study was designed to determine the distribution of HPV infections, cervical lesions and the known risk factors for cervical cancer among women living in the Akuse sub-district, Ghana.

Methods: A cross sectional study was designed with a house-based recruitment of women between the ages of 15 and 70 years. Although a single strategy was designed for reporting for specimen collection in the study, three strategies were used. The additional strategies were a result of an initial low response rate resulting from the women not making time off their daily and economic activities to attend the Hospital for specimen collection and also based on the information and suggestions from the subsequent interaction with the women. These strategies were, a) reporting to the hospital within a short time period after recruitment at home, b) reporting to the hospital within a long time period after recruitment at home and c) reporting at a location within a community after recruitment at home. Each participant had the option of providing a specimen by either or both self-specimen collection and health personnel specimen collection. Human Papillomaviruses were detected in each of the pair of the specimen by a multiplex-nested PCR amplification and genotyped by the xMAP technology.

Results: Of the 38 genotypes detected with both specimen collection methods, the following were the commonest detected with self-specimen; HPV16 (5.9%; 95% CI 3.0% – 9.0%), HPV35

(4.7%; 95% CI 2.0% – 8.0%) and HPV40 (4.7%; 95% CI 2.0% – 8.0%). The commonest detected with the health-personnel collected specimen were HPV35 (2.8%; 95% CI 1.0% – 5.0%) and HPV58 (2.8%; 95% CI 1.0% – 5.0%). A significant difference was obtained between the overall HPV prevalence determined with the self-collected specimen [43.1% (95% CI 38.0% – 51.0%)] and that with the health-personnel collected specimens [23.3% (95% CI of 19.0% – 31.0%)]. Varying reporting rates were observed for the three reporting strategies as well as varying preferences for the specimen collection methods. All the women were negative for cervical cancer and showed no dyskaryosis (intraepithelial lesion).

Conclusion: The findings of this study provide the necessary baseline data needed to contribute to the development of a cervical cancer prevention plan and indicate that the Akuse sub-district is a high risk community for HPV infection and a low risk community for cervical cancer.



CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

Cervical cancer remains a disease of global public health concern, although it is the only preventable cancer with a record of a very effective prevention method (Bradley *et al.*, 2005). As such, there are consistent efforts to reduce the high rates of incidence and mortality recorded in the World, Africa and Ghana. The WHO has consistently published its Comprehensive Cervical Cancer Control Guide Book with the aim of helping to reduce the burden of cervical cancer on women (WHO; Guideline Development Group, 2014; WHO; Chronic Diseases and Health Promotion Group, 2006). This section of the thesis presents a brief description of the background to this study by throwing light on the WHO's position and research conducted on cervical cancer prevention and control, and the challenges associated with cervical control.

According to the WHO, the goal of any comprehensive cervical cancer prevention and control programme should be to reduce the burden of cervical cancer by a) preventing and reducing HPV infection, b) detecting and treating cervical pre-cancer lesions (screening) and c) providing treatment and palliative care for those who do develop the disease (WHO; Guideline Development Group, 2014; WHO; Chronic Diseases and Health Promotion Group, 2006). The development of such a programme should start with the development of a national policy, which should include the gathering and review of country-specific empirical data to answer key questions on baseline characteristics (WHO; Guideline Development Group, 2014). These country-specific empirical data should include data on; a) the burden of HPV

infections (this includes the following prevalences; overall HPV, genotype specific HPV, age-specific HPV, multiple HPV infection, high risk HPV type, low risk HPV type, and if possible HPV genotype variants), b) the prevalence of cervical pre-cancer lesions, c) the distributions of the sub-categories of the known and globally accepted sexual and reproductive risk (behaviour characteristics) factors among women, d) the extent of and the factors that influence the use of condom (very important for all STI prevention), e) factors influencing the reporting of women for cervical cancer screening and f) the level of knowledge on cervical cancer among women; All of these data should be collected at the community level through screening activities.

In the absence of a national cervical cancer screening programme, Ghana, like other developing countries, has had challenges regarding her efforts at reducing the public health burden of cervical cancer and HPV infection through cervical cancer screening. Some of these challenges include the absence of a national guideline for cervical cancer screening and a largely opportunistic and centralised (in Accra and Kumasi) cervical screening activities (Adanu *et al.*, 2010; WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010). These opportunistic screening activities have mostly been as a result of referrals for Pap smear and VIA by Physicians (Adanu *et al.*, 2010; Adanu, 2002). A further challenge is that the Centres that provide these opportunistic cervical cancer screening services in Ghana were as a result of pilot programmes (Adanu *et al.*, 2010; Adanu, 2002), which, instead of expanding to other locations, have remained in the hospitals that hosted the pilot projects. One of the project centres, the Ridge Hospital, which is the regional hospital of the Greater Accra Region, continues to provide referral based cervical cancer screening services.

Another challenge is limited localisation of the projects; that is, the two main programme-based cervical screening and treatment service projects were conducted in Accra. One of which was sponsored by the Alliance for Cervical Cancer Prevention (ACCP) and The Johns Hopkins Program of International Education in Gynaecology and Obstetrics (JHPIEGO) (Bradley *et al.*, 2005). That project implemented visual inspection pilots screening at two sites in Accra (Abotchie and Shokar, 2009). Although it resulted in the drafting of a cervical cancer prevention and control programme, that was never implemented. The second project, also localised in Accra, was part of the Women's Health Study in Accra (WHSa), where cervical cancer screening by Pap smear testing was conducted (Duda *et al.*, 2005). These challenges have resulted in a very low coverage of cervical cancer screening in Ghana, which was estimated to be between 2.2% and 8.8%. (WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010; Abotchie and Shokar, 2009; Adanu, 2002). These data show that there is much to be done in Ghana to reduce the burden of cervical cancer.

The study reported in this thesis, in addition to Pap smear testing, used HPV DNA testing to screen women in the general population of the Akuse sub-district. That is, of the three available screening methods (VIA, Pap test and HPV DNA test) two were used in this study. Although these screening methods are discussed in section 2.9, the following is a brief discussion of the bases of the selection of HPV DNA testing for this study.

HPV DNA testing (one of the secondary prevention methods for the control of cervical cancer) is the most sensitive and reproducible of the three secondary prevention methods. It involves detecting and genotyping the HPV in collected cervical swab specimen, if any. The presence of some type of HPV (known as high risk HPV types, discussed in section 2.6) indicates a high risk for the development of cervical pre-cancer lesions and cancer, while the presence of some other HPVs (known as low risk HPV types, discussed in section 2.6)

indicate a low risk for the development of cervical pre-cancer lesions and cancer. However, the major limitation of this method is its slightly lower specificity for the detection of high grade cervical lesions, compared to the earlier developed cytology based Pap testing (Zhao *et al.*, 2012; Katki *et al.*, 2011; Zhao *et al.*, 2010; Dillner *et al.*, 2008). The central debate on improving HPV DNA testing is currently focused on the algorithm for its inclusion in primary cervical cancer screening programmes and for the triage of women (Saslow *et al.*, 2012; Luesley and Leeson, 2010; Arbyn and European Commission. Directorate-General Health & Consumer Protection, 2008). Another significant issue regarding the usefulness of HPV DNA testing in cervical cancer control is its current high cost, which is limiting its use in developing countries such as Ghana. However, the recent development of test kits such as the CareHPV kit holds a great potential for reducing the cost of HPV testing and increase its availability in developing countries. While waiting for these improvements, it is necessary for this country to generate background data of the HPV genotypes prevalent among its population and determine if there are DNA sequences differences that may be useful in the choice of HPV testing kits when they become available. This informed the HPV genotyping and the DNA sequencing of the most prevalent genotypes in this study.

Although not directly a part of the study reported in this thesis, it is worth noting that the most recent success at addressing the immense global public health burden of cervical cancer has been the production of three HPV vaccines for primary prevention of HPV infection and therefore the control of cervical cancer and the other HPV related cancers (Chatterjee, 2014; Wang and Roden, 2013; Van de Velde *et al.*, 2012; Giuliano *et al.*, 2011; Schellenbacher *et al.*, 2009; Aires *et al.*, 2006; Harper *et al.*, 2004). As more vaccines are developed, the choice of a vaccine will be informed by the data on the genotype specific prevalence in a country. For now, a divalent vaccine, known as Cervarix[®] protects against HPV-16 and HPV-18,

while the tetravalent vaccine, known as Gardasil® protects against HPV-16, -18, -6 and -11 (Dunne *et al.*, 2008). More recently, a nonavalent vaccine against HPV-16, 18, -6, -11, -31, -35, -45, -52 and -58 has been developed (Chatterjee, 2014; Van de Velde *et al.*, 2012). With the assistance of the Global Alliance for Vaccine initiative (GAVI), these vaccines have been made affordable to developing countries with demonstration project in Uganda and Tanzania. Additionally, through the Gardasil Access Programme (key initiative), the Merck-Quigen efforts and PATH support, and using a school based approach, Ghana, Guyana, Haiti, Honduras, Lesotho, Cameroon, Uganda and Tanzania, have generated a wealth of knowledge and lessons on HPV vaccination for girls between the ages of 9 and 13 years (Gulland, 2012; Quentin *et al.*, 2012; Watson-Jones *et al.*, 2012). Rwanda (in the year 2011) and Lesotho (in the year 2012) initiated a National HPV vaccination programme (Gulland, 2012; Watson-Jones *et al.*, 2012). Although Ghana is a GAVI eligible country for support there is currently no national HPV vaccination programme, however, a pilot has been initiated and the vaccines are also available in some private health facility (Coleman *et al.*, 2011; WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010).

1.2 PROBLEM STATEMENT

Cervical cancer is the fourth commonly diagnosed and the fourth most fatal cancer among women worldwide, with an estimated 528,000 new cases and 266,000 deaths in the year 2012 (GLOBOCAN, IARC, 2012). It is common among women within the economically active age group; between the ages of 30 years and 55 years (GLOBOCAN, IARC, 2012; Jemal *et al.*, 2011). Developing countries, particularly African countries and the countries of the sub-Saharan Africa region, continue to bear the highest proportion of the global burden of cervical cancer (GLOBOCAN, IARC, 2012; Jemal *et al.*, 2011) and cervical pre-cancer

lesions among women in the general population (Clifford *et al.*, 2005a, 2003). The extent of which was shown by the high estimated incidence rate per 100,000 women of 42.7 for Eastern Africa, 33.37 for Melanesia, 31.5 for Southern Africa, 30.6 for WHO Middle Africa region (Angola, Cameroon, Central Africa Republic, Chad, Congo Brazzaville, Congo, Equatorial Guinea and Gabon) and 28.7 for Western Africa; it is interesting to note that these are the five highest in the world (GLOBOCAN, IARC, 2012). Furthermore, cervical cancer was the leading cause of cancer death among women in Africa, with an estimated 56,601 (22.6%) deaths in the year 2012 (GLOBOCAN, IARC, 2012). For Ghana, the fact that cervical cancer was the leading contributor to the burden of cancers diagnosed in Ghana (GLOBOCAN, IARC, 2012; WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010) makes it a critical public concern. Specifically, of the 6.57 million Ghanaian women (aged 15 years or older) estimated to have been at risk of cervical cancer, 3052 new cases were estimated to have occurred in the year 2012; this was about 19.3% of all estimated cancers in Ghana (GLOBOCAN, IARC, 2012; WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010). Additionally, it was estimated to have been the leading cause of cancer death in Ghana, with an estimated 1556 deaths (14.6% of all cancer deaths) in the year 2012 (WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010). Although there is paucity of published data or reports on cervical cancer at the regional and district level in Ghana, few studies that achieved low screening coverage, have indicated low prevalences of cervical pre-cancer lesions (Adanu *et al.*, 2010). In one such study in rural Accra, a prevalence of between 4.40% -5.88% for cervical pre-cancer lesions by VIA was reported (Bradley *et al.*, 2005). In the Ashanti region, prevalences of 3.5% in the Nkawie community and 12.6% in the Agogo community, both determined by Pap testing have been reported (Handlogten *et al.*, 2014).

As has been globally identified, cervical cancer is a multifactorial and multistage disease that develops over a long period of time between 10 and 20 years (WHO; Chronic Diseases and Health Promotion Group, 2006; Sankaranarayanan *et al.*, 2003; Sellors *et al.*, 2003). It is caused by the persistent infection of genotypes of HPV, whose acquisition is mainly the result of the sexual and reproductive behaviour of women and to some extent, the sexual behaviour of their male sexual partners (Franco *et al.*, 1999; Walboomers *et al.*, 1999). These characteristics are the factors that predispose a woman to acquire an HPV infection and enhance the persistence of the HPV infection. It is this persistent HPV infection that causes cervical pre-cancer lesion and cervical cancer; necessarily, the persistent HPV infection of the cervix by some genotypes of the Human Papillomavirus (HPV) is required, but it is not sufficient alone to cause cervical cancer (Franco *et al.*, 1999; Walboomers *et al.*, 1999). In other words, the persistence of an HPV infection in addition to the continued involvement in these same sexual and reproductive characteristics leads first to the development of low grade cervical pre-cancer lesions, which may progress to high grade pre-cancer lesions and then to cervical cancer. Each of these, progressions is influenced by the continuous persistence of the HPV infection and continuing participation in the sexual and reproductive behaviours. Due to the fact that cervical cancer screening coverage is low and has largely been opportunistic in Ghana, data on these risk factors and cause of cervical cancer among the general population are limited. In other words, there are limited community data on sexual and reproductive risk characteristics, HPV infection and cervical pre-cancer lesions in Ghana (have not identified communities at high risk) and these are hampering the control and prevention of cervical cancer by ensuring the persistence of a high burden of incidence, prevalence and mortality due to cervical cancer in Ghana.

The consequence of cervical cancer on the nation is evident by the fact that cervical cancer is common among women within the economically active age groups. As such it reduces the national productive work force, which in the long run will affect the development of the Ghana. The role of women in Ghana's informal economy is widely acknowledged, as most of the commercial trading activities are largely controlled by women. Additionally, the role of women in the formal sector of the Ghanaian economy is growingly becoming stronger by the day and therefore losing women to cervical cancer would hamper the development of Ghana. Furthermore the cost of scaling-up cryotherapy treatment for women diagnosed with cervical precursor lesion in Ghana was estimated to cost the nation between GH¢ 11,214,728.00 and GH¢ 7,850,309.00 if screening is performed every five years (Quentin *et al.*, 2010). The implication of cervical cancer to the family and community is similarly disturbing, because this same age range of women are mostly mothers of young families and sometimes contribute to the finances of the family. Therefore, losing women to cervical cancer may affect the development of children, and the stability of the family. For the individual woman who is diagnosed with cervical cancer, the cost of health care and the need for support are often issues that are burdensome. For example, the costs of screening ranged from GH¢ 7.30 to 21.86 (US\$ 4.93 to 14.75) for VIA, and from GH¢ 70.04 to 125.19 (US\$47.26 and 84.48) per woman treated with cryotherapy in the year 2010 (Quentin *et al.*, 2010). The purpose of the study reported in this thesis was to contribute the critically needed empirical data to inform Ghana's cervical cancer prevention and control planning and implementation.

1.3 CONCEPTUAL FRAMEWORK

The previous section described the biological factors interacting to result in the development of cervical cancer and this is shown in figure 1.1. While not one of such biological risk

factors for cervical cancer, the lack of screening by women (either due to limited availability or lack of interest or knowledge) is another factor contributing to the high burden of cervical cancer and cervical pre-cancer lesions in developing countries (Scarinci *et al.*, 2010a; Lowy *et al.*, 2008; Mayrand *et al.*, 2007a).

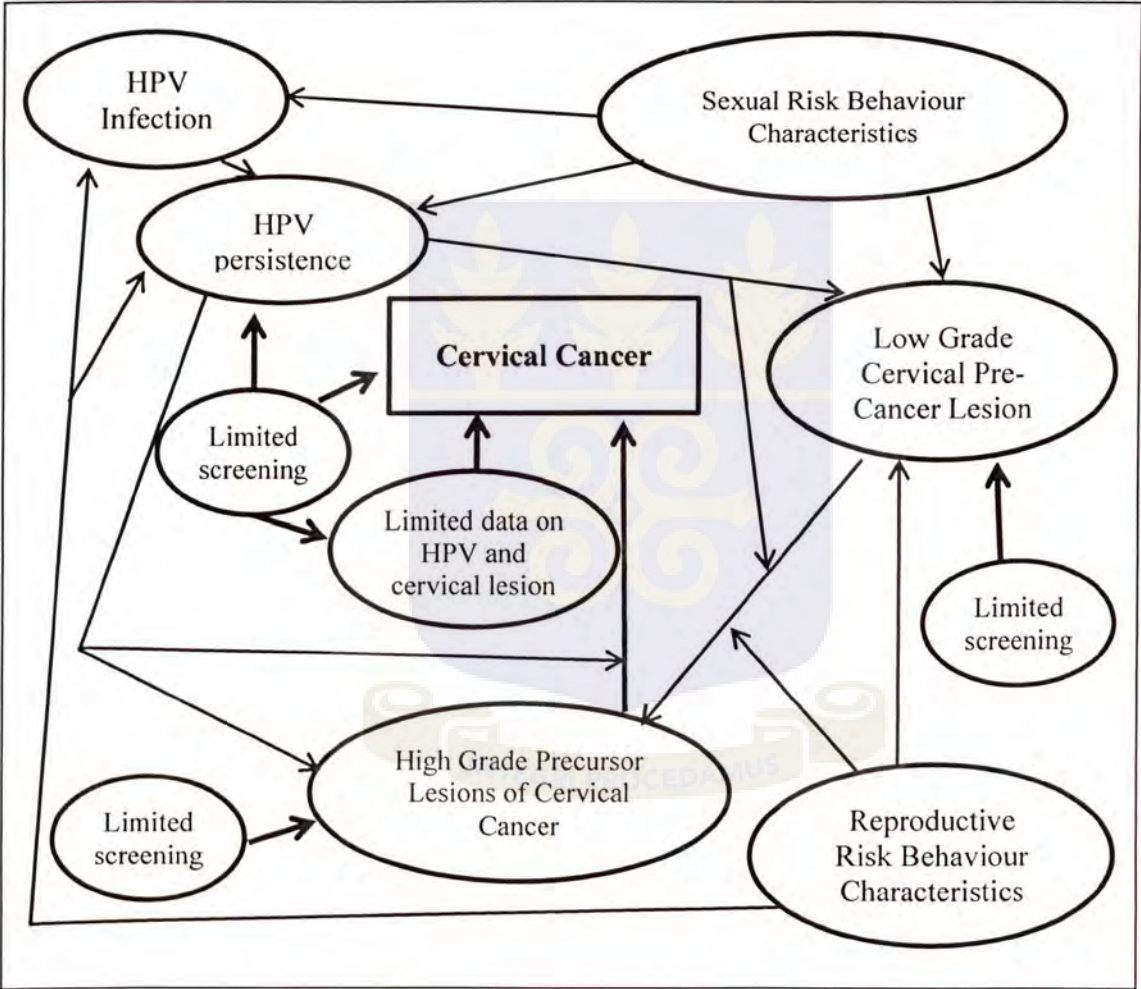


Figure 1.1: Conceptual framework of cervical cancer and related risk factors.

This is because screening ensures early detection of low grade and high grade lesions and affords timely treatment to avert the progression of these precursor lesions to cervical cancer. Additionally, cervical screening, particularly using HPV testing afford the opportunity to avoid persistence of HPV infection. Furthermore, screening ensures the availability of the necessary data on the prevalence of HPV infection and related pre-cancer lesions as well as of the distribution of sexual and reproductive risk behaviour characteristics for the planning of prevention and control of cervical cancer. Therefore, by providing community based cervical screening, information on the sexual and reproductive behaviour characteristics, HPV infection and cervical pre-cancer lesions among the general population, which are equally significant concerns to the health of women, will be made available.

1.4 RESEARCH QUESTIONS

1. As suggested by the prevalence of STIs in the Lower Manya Krobo District, can the communities of one of its sub-districts, Akuse, be said to be communities at high risk for HPV infection based on the distribution of the sub-categories of each of the known sexual risk factors for HPV infection and if so, will this high risk status be confirmed by the prevalence of HPV infection determined in this study? Also, can the communities of this sub-district be said to be high risk for cervical lesion based on the distribution of the sub-categories of each of the known risk factors for cervical lesion development and if so will this be consistent with the determined prevalence of cervical lesion for the sub-district?
2. Will the HPV infection burden in the sub-district be as high as has been reported in a similar study in Nigeria (Thomas *et al.*, 2004), the only large cervical cancer and HPV study in West Africa at the time of the design of this study?

3. Will the data on the burden of HPV infection determined with self-collected specimen be as comparable, if not higher as expected than those determined with specimen collected by health personnel?
4. Will the prevalence of cervical precursor lesions in the sub-district be as high as may be expected based on the Globocan estimates for Ghana in the year 2012?
5. Are the demographic, sexual and reproductive characteristics of the study population associated with their use of condom and their reporting status for specimen collection?
6. Are the variants of HPV16, HPV 18 and HPV 45 in the Akuse sub-district of the African lineage and how similar are they to those detected in other African countries?

1.5 RATIONALE

In view of the fact that the distribution of HPV genotype infections varies greatly across populations (Castle *et al.*, 2012; de Sanjosé *et al.*, 2007), HPV screening needs to be performed in every geographical region in order to implement and evaluate the effect of suitable vaccination strategies. Particularly in low-resource countries like Ghana, there is the need to determine population/community-based HPV infection prevalences, validate low-cost, simple, and accurate HPV tests and develop strategies to encourage women participation in screening programmes. Although it was estimated that over 6.5 million women in Ghana were at risk of cervical cancer in the year 2012 (GLOBOCAN, IARC, 2012), Ghana continues to lack the important country-specific empirical data (at the community level) on HPV infection, cervical lesions and risk factors for cervical cancer needed for prevention control planning (as indicated in section 1.1). As such, Ghana does not have a cervical cancer prevention and control plan which is based on Ghanaian-specific empirical data, as

encouraged by the WHO. Specifically lacking are the population based assessments of the burden of overall HPV infection, genotype-specific HPV infection prevalences, age-specific HPV prevalence and the proportion of multiple HPV infections. Furthermore, the lack of data on the distribution or profile of the known/accepted risk factors (sexual and reproductive behaviour characteristics), the extent of and the factors that influence the consistent use of condom and overall prevalence of cervical pre-cancer lesions are limiting the identification of communities at high risk. The current state of the lack of data and issues related to prevention of cervical cancer in Ghana is depicted in figure 1.2.

With these data, Ghana will be able to develop a well informed and effective cervical cancer prevention and control plan, which will enable the achievement of the following; a) identify Ghanaian communities at high risk for HPV infection and cervical cancer, b) make the appropriate choice of screening methods to use, d) make the appropriate choice of vaccine to use, e) make efforts to increase the number of women participating in cervical screening activities, f) identify needs/resources for cervical screening, g) plan for and provide resources where needed, and h) prioritize based on need and risk status in relation to the selected screening method. These will result in a reduced incidence and increase early detection of cervical cancer and thereby result in a low mortality rate due to cervical cancer in Ghana, which is currently high (GLOBOCAN, IARC, 2012). Additionally, these HPV data are important in the selection of HPV vaccine, selection of HPV test kits and the future evaluation of the benefits of HPV vaccination as a primary prevention of cervical cancer. Obtaining these HPV data will be effectively facilitated by the knowledge of which specimen collection methods (self-collection or health personnel collection) is more acceptable to women, more defining of the women's risk of development of cervical lesions based on HPV

infection, and which screening methods (HPV test, Pap test or VIA test) the women in Ghanaian communities may prefer.

Since HPV is the commonest sexually transmitted infection (STI) and that infections by the following STIs, *Chlamydia trachomatis* (CT), herpes simplex virus type 2 (HSV-2) and HIV (Muñoz *et al.*, 2006; Samoff, 2005; Smith *et al.*, 2002a, 2002b, 2004), encourage the development of cervical lesion, HPV infections and persistence (Burchell *et al.*, 2006; Samoff, 2005), this district was regarded as a potential high risk for HPV infection because it was the district with the highest rates of the most common Sexually Transmitted Infection in Ghana in 2010 (at the time of the design of the study).



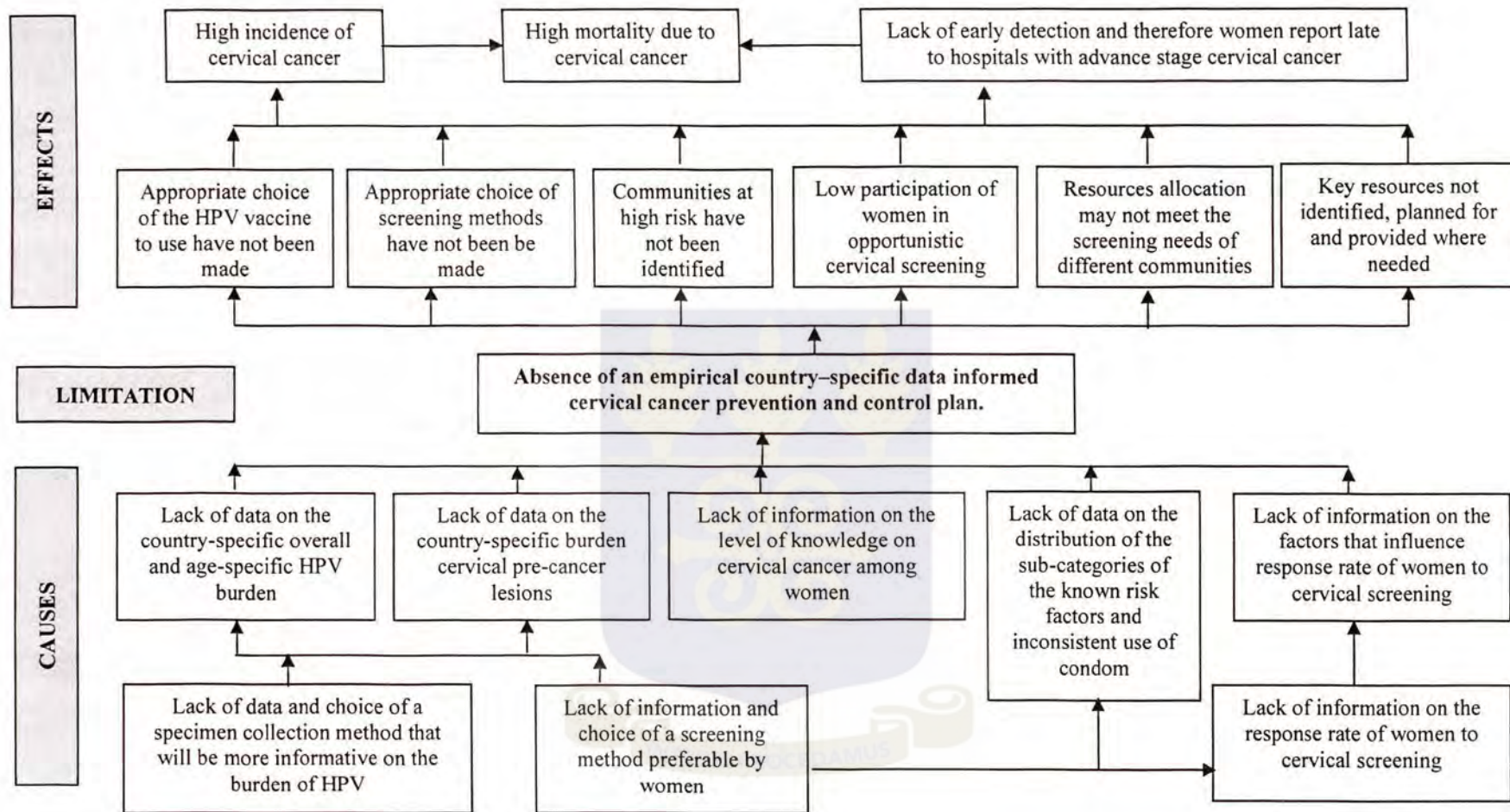


Figure 1.2: The current status of the relationships between the country-specific data needed to develop a cervical cancer prevention plan in Ghana.

1.6 GENERAL OBJECTIVE

To determine the prevalence of cervical pre-cancer lesions and HPV infection and their associated risk factors among women living in Akuse sub-district in Ghana.

1.6.1 Specific Objectives

1. To determine the proportion of women at risk of HPV infections and cervical pre-cancer lesions positivity based on the distributions or profiles of the accepted risk factors among the women. In order to ascertain the community-risk status.
2. To determine the difference in HPV prevalences between analysis of self-collected specimen and health personnel collected specimen.
3. To assess the level of agreement and concordance between self-collected and health personnel collected specimen for HPV genotype detection.
4. To determine the variants of the detected oncogenic HPV16, 18 and 45 genotypes present in the study population.
5. To determine the prevalence of cervical pre-cancer lesions.
6. To determine participant characteristics which influence the reporting of the women for cervical cancer screening specimen collection
7. To determine the factors influence the use of condom, presence of cervical lesions and HPV positivity

1.6.2 Hypotheses

Since there was no report of a study conducted in Ghana in respect of determining the burden of HPV infection and cervical lesions at the community level, as at the time of this study, the hypotheses of this study was based on a study designed in a similar manner by Thomas *et al.*, 2004. This was the major community based study on cervical cancer and HPV screening in West Africa as at the time of the design of the study reported in this thesis. That study reported an overall HPV prevalence of 26.3% and an odds ratio of 3.0 for the association of HPV positivity with cervical pre-cancer lesions. Therefore, the hypotheses of this study were as follows;

1. The prevalence of HPV infection as determined with the health personnel collected specimen of women in the Akuse sub-district of the Lower Manya Krobo district in the Eastern Region of Ghana was greater than 26.3%, as reported in the community-based study of HPV and cervical pre-cancer lesions in Nigeria (Thomas *et al.*, 2004).
2. Women, living in the Akuse sub-district of the Lower Manya Krobo district in the Eastern Region of Ghana, who were infected with an HPV (exposure) were three times more likely (odds ratio of 3.0) to test positive for cervical pre-cancer lesion (outcome) compared to women who were not infected with an HPV.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE CERVIX UTERI

The principles and application of cervical screening and related methods that are discussed later in this thesis would be better understood with basic description/knowledge of the anatomy of the uteri cervix. This description is provided in this section. This part of the female reproductive organ is the narrower and lower part of the uterus (about 3 - 4 cm) that extends into the vagina (LEPPERT, 1995). Although the shape and size of the cervix is influenced by the age, hormonal status, and parity of the woman, it is usually cylindrical or conical in shape (Figure 2.1) (WHO; Chronic Diseases and Health Promotion Group, 2006).

The outer half of the cervix extends into the vagina (2.5 – 3.5 cm in diameter) and is the part that is visible during speculum examination. The opening of the cervix into the vagina, the external os, is seen as a centrally placed small round opening in women who have not given birth via the vagina, but as an irregular slit after childbirth *per vaginam*; the area external to this opening and that forms the intravaginal portion of the cervix for girls before puberty and is exposed to the vaginal secretions is the ectocervix. The external os leads into the upper half of the cervix, through the cervical canal and ends at the internal os. The upper half of the cervix that is not seen during a speculum examination is referred to as the endocervix and is traversed by the cervical canal (WHO; Chronic Diseases and Health Promotion Group, 2006; Sankaranarayanan *et al.*, 2003).

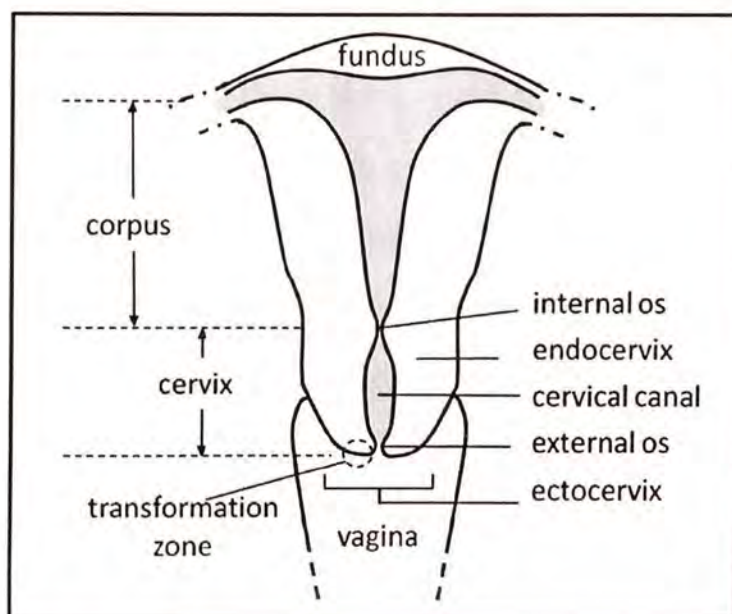


Figure 2. 1 Diagram of the uterus of a woman within her reproductive age.

Adapted and modified from comprehensive cervical cancer control WHO, 2006

The lining of the cervix is made up of two types of epithelia, each at a different location. The stratified squamous epithelium covers most part of the ectocervix, extending towards the vagina and similar to that found in the vagina. This consists of layers of flattened cells, which are mainly protective. The other, the columnar (glandular) epithelium, forms the lining of the cervical canal and some part of the ectocervix closer to the external os. This consists of a single layer of rectangular shaped mucus secreting cells. The junction between these epithelia, which develops from birth to puberty as the uterus and cervix increase in size, referred to as the original squamocolumnar junction (OSCJ), forms a sharp visible line (Sankaranarayanan *et al.*, 2003).

After puberty and during the reproductive period, the growth of the female reproductive organ results in the outward extension of the endocervical canal on to the ectocervix,

exposing the columnar epithelium to the acidic condition of the vagina and pushing the original SCJ outwardly away from the external os. The exposed columnar epithelium gets gradually replaced by new stratified squamous epithelium in a process referred to as squamous metaplasia; resulting in a new squamocolumnar junction (NSCJ); depending on the woman's age, hormonal status, birth trauma, pregnancy status and use of oral contraceptives, the NSCJ is at varying location from the external os (Sankaranarayanan *et al.*, 2003). The area between the original and new SCJ is termed the transformational zone (TZ) (Sankaranarayanan *et al.*, 2003; Sellors *et al.*, 2003). During the collection of specimen for cervical cancer testing, it is the ectocervix, TZ (where about 90% of cervical cancer occur and the mainly sampled location) and the endocervix that are sampled.

2.2 CANCER

In simple terms, cancer develops when cells divide/multiply without cellular control and progressively acquire other traits (known as the hallmark capabilities) which make them able to evade cell death mechanisms and eventually outgrow the surrounding cells and form malignant tumours. These cells may spread and invade other normal tissues of the organism, destroying the tissue (Hanahan and Weinberg, 2011; WHO; Chronic Diseases and Health Promotion Group, 2006; Hanahan and Weinberg, 2000). The resultant disease is generally referred to as cancer, however, based on the tissue or organ from which the uncontrolled cell division arose, a type of cancer is defined; there are over a 100 types of cancers identified in humans. Of these, the cancer of the cervix uteri is common worldwide (Ferlay *et al.*, 2010) and is the primary interest of the study reported in this thesis.

2.3 BURDEN AND EPIDEMIOLOGY OF CERVICAL CANCER

Although cervical cancer is common among women in the active working age group of 35 years to 59 years, there are clear regional and both inter and intra country variation in the incidence and mortality rates of this cancer. For instance, while cervical cancer incidence and mortality have been reducing since the late 1950s in developed countries, these have been increasing in developing countries, primarily because of the introduction of an effective and quality screening programmes with Pap smear testing in only developed countries (WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010). The following sub-sections describe the extent of these variations in incidence and mortality and thereby emphasising the need for each country or population to determine its specific burden and epidemiology to inform its cervical cancer prevention and control activities.

2.3.1 Variation in the Incidence of Cervical Cancer

The estimated global incidence of cervical cancer in the year 2012 according to the data publish by GLOBOCAN was 527,624 cases, with an age-standardized incidence rate (ASR) of 14.0 per 100,000 women per year, making it the seventh most common cancer overall and the fourth most common cancer among females, after breast, colorectal and liver cancers. This burden has continuously been excessively high among developing countries, such that in the years 2002, 2008 and 2012, 83%, more than 85% and 84.3% respectively of the global burden of cervical cancer were in developing countries (GLOBOCAN, IARC, 2012; Jemal *et al.*, 2011; Ferlay *et al.*, 2010; GLOBOCAN, IARC, 2008; Parkin *et al.*, 2005). Furthermore among developing countries, a variation is seen that indicates that a highly disproportionate burden of the incidence of cervical cancer had been in Africa (GLOBOCAN, IARC, 2012; Jemal *et al.*, 2012). This was evident by the estimated 80,400 new cases of cervical cancer in the year 2008 and 92,000 new cases of cervical cancer in the year 2012 (GLOBOCAN,

IARC, 2012; Jemal *et al.*, 2012). In addition to this global regional variability in incidence, regional level variability in Africa has also existed. While countries in the WHO Eastern Africa region continued to bear the highest estimated burden of cervical cancer globally, with estimates of age-standardized incidence rate (ASR) of 34.5 per 100,000 women in 2008 and 42.7 per 100,000 women 2012, countries in, the WHO Western Africa region bore the second highest estimated globally burden in 2008, with an estimated incidence ASR of 33.7 per 100,000 women and the fifth highest burden in 2012, with an estimated ASR of 29.5 per 100,000 women. Furthermore, the countries of the WHO Southern Africa region were estimated to have recorded the third highest burden of cervical cancer in 2008, with an incidence ASR of 26.8 per 100,000 women and the third in 2012, with an estimated incidence ASR of 31.5 per 100,000 women (GLOBOCAN, IARC, 2012; Jemal *et al.*, 2011; Ferlay *et al.*, 2010; GLOBOCAN, IARC, 2008; Parkin *et al.*, 2005).

The estimated high burden of cervical cancer reported for the WHO Africa regions, as described in the previous paragraph was also distributed varyingly at the country level (GLOBOCAN, IARC, 2012; Jemal *et al.*, 2011; Ferlay *et al.*, 2010; GLOBOCAN, IARC, 2008; Parkin *et al.*, 2005). For instance, country-specific estimates of cervical cancer incidence, reported as age-standardised rates per 100,000 women, in 2012 for countries of the Eastern African Region ranged between 15.0 in Mauritius and 75.9 in Malawi (GLOBOCAN, IARC, 2012). Countries of the WHO Middle Africa Region had estimates ranging from 18.8 per 100,000 women for the Chad to 35.5 per 100,000 women for Angola. The lowest and highest estimates in the Northern African Region were 2.3 per 100,000 women for Egypt and 31.1 per 100,000 women for the Western Sahara (GLOBOCAN, IARC, 2012). In the Southern Africa Region, Namibia and Swaziland were expected to have the lowest and highest estimates, 14.7 and 53.1 per 100,000 women respectively. The estimates of the ASR

per 100,000 women for countries in the Western Africa Region ranged between 8.6 in Niger and 44.2 in Mali. The ASR estimates for Ghana (35.4 per 100,000) was higher than those of its close neighbours; Nigeria (29.0), Benin (27.6), Togo (21.5), Burkina Faso (23.3), and Cote d'Ivoire (21.7). This converts to 3052 new cases in the year 2012, accounting for 35.4% of all cancers in Ghana (GLOBOCAN, IARC, 2012).

It is worth noting that these country-specific estimates for Africa were based on very few empirical data from mostly cancer registries in mostly one or two major cities of some of the countries. For example, countries such as Ghana, where there were no empirical cancer registry data, estimates were obtained by modelling data from the neighbouring countries; Mali, Nigeria, and Cote d'Ivoire. Also, the difference between the GLOBOCAN 2012 estimates and those of its earlier versions, do not indicate a time trend change, since the few data and modelling methods used in obtaining these estimation had been changing significantly each time for most countries, including Ghana (GLOBOCAN, IARC, 2012; Jemal *et al.*, 2012; WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010; GLOBOCAN, IARC, 2008).

In addition to the geographical variability in the incidence of cervical cancer discussed so far, there is also variation in the burden of cervical cancer with the age of women. Generally, cervical cancer is common among women within the working age group (Gustafsson *et al.*, 1997). Data that show the worldwide age-specific incidence rates indicated that cervical cancer generally increased with age, but there is a sharp rise between the ages of 35 years and 54 years and a peak between 50 years and 55 years (Parkin *et al.*, 2005). However, country-based age-specific incidence varied significantly and this variation has been influenced by the availability of quality of screening programmes in developed countries (WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010).

The variability in the incidence of cervical cancer within Ghana is not currently determinable because the few studies on cervical cancer thus far have been centralised and hospital based. For instance in one such study at the Korle-Bu Teaching Hospital, where most of the women were referred from almost every part of the nation, it was observed that 58.3% of the reported cases of gynaecological cancers were cancers of the cervix, with a prevalence of 1.6% (Nkyekyer, 2000). on the other hand, the only community-based study, which was in Accra, reported a 0.6% prevalence for high grade squamous intraepithelial lesion but did not detect any case of cervical cancer (Grace *et al.*, 2006).

2.3.2 Variation in the Mortality of Cervical Cancer

The global burden of the mortality due to cervical cancer, just like that for incidence, was the fourth leading cause of cancer death among females in the years 2012, and it was estimated that 265,653 deaths had occurred in that year, resulting in a mortality to incidence ratio of 50.3%. As was observed with the global incidence of cervical cancer, there were geographical variation in the distribution of the burden of cervical cancer mortality (GLOBOCAN, IARC, 2012). Specifically, while cervical cancer mortality rates in developed countries have been significantly low, the rates have been high in developing countries (GLOBOCAN, IARC, 2012; WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010). Furthermore, although developing countries carry the highest burden (about 87%) of cervical cancer deaths; there are shape variations between these developing regions of the world. For instance, 144,434, 60,098 and 28,565 of the global deaths due to cervical cancer were estimated to have occurred in Asia, Africa and Latin America/the Caribbean respectively in the year 2012. Once again, the high mortality burden (age-standardized rate) of 17.5 per 100,000 women per year reported for Africa, (cervical cancer was the second to breast cancer death among females) was varyingly distributed among the

WHO Africa regions. For example,, the estimated, age-standardize mortality rates per 100,000 women per years of 27.6, 22.2 and 20.7 for the WHO Eastern Africa, WHO Middle Africa Regions and Melanesia accounted for the three highest mortality rates due to cervical cancer worldwide in 2012, while that for the Southern Africa Region was 17.9 (GLOBOCAN, IARC, 2012). Worldwide age-specific mortality rates of cervical cancer showed an increase with a sharp peak at age 44 years and then a decline after 50 years.

Country level variations in the projected estimates of mortality were also evident. Specifically, 7 out of the 17 countries in the Eastern Africa Region were expected to record high rates of between 32.4 and 49.8; notable among these were Malawi, Mozambique and Comoros, while 2 of the 17 were expected to record low rates of below 10.0. In the Western Africa Region, Mali was expected record the highest mortality per 100000 women of 31.2 while Ghana was expected to have record the seventh highest mortality of 18.9; which was higher than those of its neighbouring countries; Cote d'Ivoire (14.7), Burkina Faso (18.6), Togo (13.8), Nigeria (17.5) and Benin (17.8) (GLOBOCAN, IARC, 2012).

Although there are not enough data to determine the variability of cervical cancer mortality within Ghana over a long period of time, an earlier 10-year review of autopsies and hospital mortality for the period 1990 and 2000, showed cervical cancer as the fourth (mortality ASCAR of 8.47) leading cause of cancer deaths in Ghana (Wiredu and Armah, 2006). However, it must be noted that the availability of treatment and the state of the cancers at reporting to the hospital are worse for most of the other cancers compared to cervical cancer in Ghana. Additionally, relative to other cancers reported in Ghana, cervical cancer was estimated to be the leading cause of death in 2012 with an annual crude mortality rate per 100,000 females of 18.9; amounting for an estimated 1556 of the cancer related deaths in

Ghana (WHO/ICO Information Centre on Human Papillomavirus and Cervical Cancer, 2010).

2.3.3 Summary of Burden of Cervical Cancer

The burden (incidence and mortality) of cervical cancer is high and highest among developing countries. Data on the epidemiology of cervical cancer varies geographically both between and within global regions, continental regions and countries, so much so that for each country to be able to plan effectively to prevent and control cervical cancer, there is a critical need to determine country specific empirical data, since data such as published by Globocan are based on statistically modelled estimates and so are not a complete definition of the state in a country. The need to develop a cervical cancer prevention and control plan for Ghana (if not a control programme) is further stressed by the high burden of cervical cancer in the West Africa region and Ghana in particular, the study reported in this thesis aimed to provide most of these baseline data needed for the development of a cervical cancer prevention plan for Ghana.

2.4 THE NATURAL HISTORY OF CERVICAL CANCER

This section on the natural history of cervical cancer presents an overview of the stages in the development of cervical cancer. A fair description and understanding of this makes the appreciation of data collected in this study much easier, since it informed the collection of specimen, identification of known risk factors and the interpretations of the results of cervical screening activities. For Instance, it is known that most neoplasia and oncogenic influence (between 80% and 90% of cervical cancers) occur in the transformation zone due to its high turnover of cells. The neoplasia arising from the squamous cells in this zone and are referred to as squamous cell carcinoma. The others, approximately 12.0% and 4.0% of cervical cancer

are adenocarcinomas and adenosquamous carcinoma respectively. These develop in the glandular, mucus-secreting surface cells of the cervical canal leading into the uterus (The International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Wang *et al.*, 2004; Shingleton *et al.*, 1995). These facts informed the collection of three cervical specimens from the TZ, external os and endo-cervix during for the Pap smear and HPV testing in this study.

Within the normal transformation zone, where the columnar cells of the epithelia are being replaced, the cells of the epithelia go through a series of processes including a cell maturation process. When this process is disrupted, mostly by HPV infection, lesions develop that have the potential of malignancy, referred to as dysplastic lesions; these well-defined lesions are mostly transient (McCredie *et al.*, 2008). Therefore, the detection of a lesion in a cross-sectional study only defines a potential risk for cervical cancer. However, due to HPV infection and persistence, as a results of participation in high risk sexual behaviour, a few of these lesions may progress to higher grade lesions, and only some of the higher grade lesions may progress to invasive cervical cancer when they are not effectively treated (McCredie *et al.*, 2008). These mean that sexual risk behaviours are risk factors for the acquisition and persistence of HPV infection; HPV infection and reproductive risk behaviours are risk factors for the development and progression of cervical pre-cancer lesions; cervical pre-cancer lesions are a risk factor for the development of cervical cancer. The roles of the sexual and reproductive risk behaviours and that of HPV in the development of cervical cancer are discussed more detail in sections 2.5 and 2.7 respectively.

This continuous increase in the severity of the precursor lesions (progression) may take between 10 and 30 years to culminate in cervical cancer, providing a long window of opportunity for detection by screening and for treatment to prevent the development of cervical cancer. These precursor lesions have been characterised and described over the years

by different terminologies, with some level of overlaps, and these are described in the next sub-section (WHO; Chronic Diseases and Health Promotion Group, 2006; Sankaranarayanan *et al.*, 2003; Sellors *et al.*, 2003).

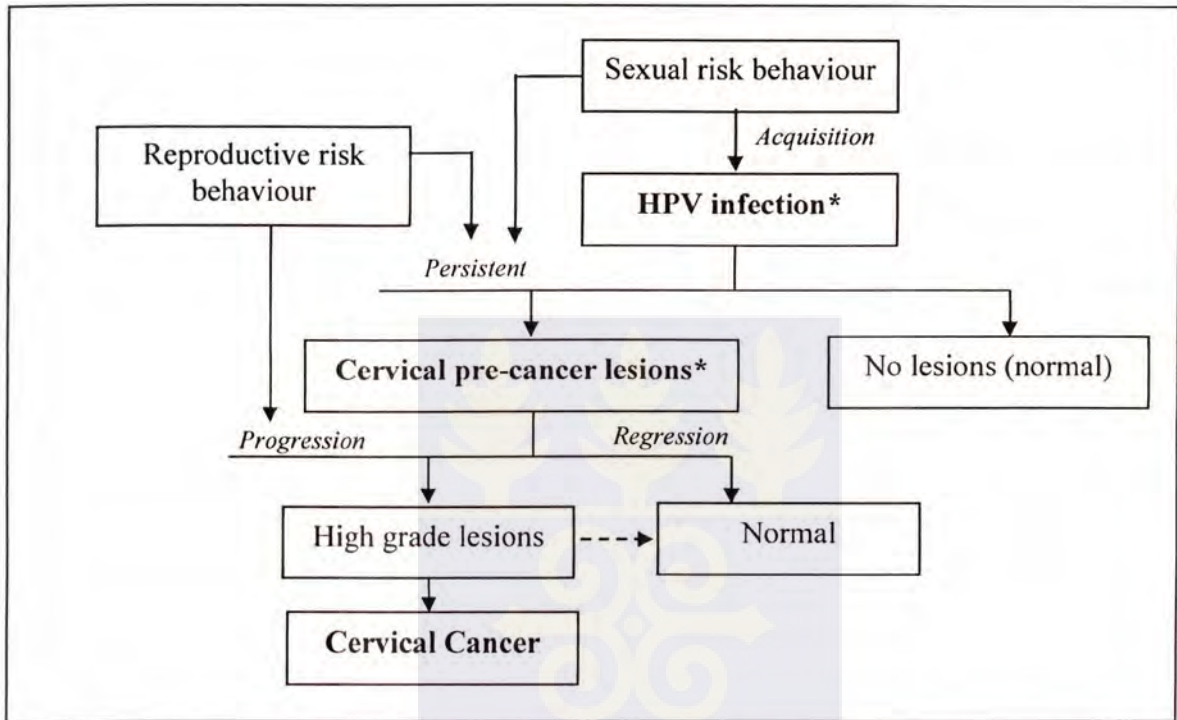


Figure 2. 2: An illustration of the multifactorial and a trifocal outcome model involved in the development of cervical cancer.

Bolden text indicates outcomes, however, two of the three (*) are also risk factors leading to cervical cancer. The broken line arrows indicate very less likelihood of occurrence.

2.4.1 Precursor Lesions of Cervical Cancer

Over the years, different cytology and histology based systems have been developed and improved for the description of cervical lesions (Table 2.1) (WHO; Chronic Diseases and Health Promotion Group, 2006; Sankaranarayanan *et al.*, 2003; Sellors *et al.*, 2003).

For instance two of the histology classification (based on the appearance of tissue section) systems use the following terminologies,

1. The dysplastic classification with grading consisting of normal, atypia, mild, moderate, severe *carcinoma-in-situ* and invasive carcinoma
2. The cervical intraepithelial neoplasia (CIN) classifications with grading consisting of grade 1 (CIN 1), grade 2 (CIN 2), grade 3 (CIN 3) and invasive carcinoma.

for cytology (based on the appearance of cells), the first classification system was the Papanicolaou classification, however, its use was limited because it lacked specificity and was not in line with the known biology of cervical neoplasia. Following this, the British developed their own cytology classification system which used the term dyskaryosis as the cytological equivalent of the histologic term dysplasia. Other systems such as the Bethesda classification with grading consisting of negative, atypical squamous cells of undetermined significance (ASC-US) or atypical glandular cells of undetermined significance (AGC-US), low-grade squamous intraepithelial lesion (SIL), high-grade SIL and invasive squamous /adenocarcinoma carcinoma, have been developed and improved (WHO; Chronic Diseases and Health Promotion Group, 2006; Sankaranarayanan *et al.*, 2003; Sellors *et al.*, 2003). The term atypia was used by all classification systems without strict criteria making studies difficult to compare.

2.5 RISKS FACTORS OF CERVICAL CANCER

As indicated in section 2.4 sexual risk behaviours and reproductive risk behaviours are the factors that following an HPV infection, predict the development and progression of cervical lesion to cervical cancer (Picot *et al.*, 2012; Shields *et al.*, 2004; Bosch *et al.*, 2002; Walboomers *et al.*, 1999). This section describes each of the categories of the sexual and

reproductive risk behaviours, which have been shown as risk factors for cervical cancers globally. The sections also describe the sub-categories of each risk behaviour showing which are high risk and which are low risk. This is useful for this study because, small cross-sectional studies, such as the one reported in this thesis, are not appropriate for identifying these characteristics as risk factors (or correctly quantify the association), primarily because of their small sample sizes are often not enough (not powered enough). Often large longitudinal and case-control studies, multi-centre studies or meta-analysis of data of a number of related studies (The International Collaboration of Epidemiological Studies of Cervical Cancer, 2007) are needed to identify each of these behaviours as risk factors within a population (Bosch *et al.*, 2002; Walboomers *et al.*, 1999). In such small sample-sized cross-sectional studies, the distribution of the participants among the low and high risk sub-categories of each of these risk behaviours becomes a good indicator of the likelihood of a related outcome (Erickson *et al.*, 2013; Castellsagué and Muñoz, 2003a). For example, if most of the participants in a relatively small cross-sectional study report an age at first sexual intercourse of less than 20 years (high risk sub-category), this will imply that that population is most likely going to be at a high risk of HPV positivity. This mode of assessment was used in this study to determine if the Akuse sub-district was high-at-risk for HPV infection and then confirmed by the results of the HPV test.

2.5.1 Sexual Risk (Behaviours) Factors

2.5.1.1 Number of Male Sexual Partners

The current and life-time numbers of male sexual partners of a woman, have consistently been shown to be risk factors for acquiring an HPV infection. Most importantly, these factors have shown the strongest associations as independent risk factors for the acquisition of HPV infection (Lenselink *et al.*, 2008) and for the development and progression of precursor

lesions to invasive cervical cancer in most populations and among different age groups (The International Collaboration of Epidemiological Studies of Cervical Cancer, 2007). Specifically, there two sub-categories of these risk factors; those who have had less than 5 and those having had 5 or more; the latter is the high risk sub-category and have recorded significant odds ratio and relative risk greater than 1.6, This odds ratio increases with increase in the number of both current and life-time number of sexual partners (Erickson *et al.*, 2013; International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Shields *et al.*, 2004). Therefore, if a majority of the participant in this study report 5 or more current or life-time male sexual partners, then the community will be high risk for HPV infection.

2.5.1.2 Age at First Sexual Intercourse (AFSI)

Early age of first sexual intercourse is also one of the strongest risk factors for both the acquisition of HPV infection and the progression to high grade lesion in most studies (Erickson *et al.*, 2013; Bahmanyar *et al.*, 2012; Berrington de González *et al.*, 2004). There are two sub-categories of AFSI, these are ages less than 20 years (sometime less than 18 years) and ages 20 years and older. The former (< 20 years or younger AFSI), is the high risk sub-category and is associated with a high risk for acquisition of an HPV infection; ages less or equal to 17 years showing the highest association with significant odds ratio (ORs) ranging between 1.8 and 2.9 (Erickson *et al.*, 2013; Bahmanyar *et al.*, 2012; Berrington de González *et al.*, 2004). However, in some age limited cross-sectional studies and in some atypical populations (Johnson *et al.*, 2012; Smith *et al.*, 2002a; Lenselink *et al.*, 2008), AFSI was not significantly associated with the risk of HPV acquisition. For one of those studies, a derived risk factor, defined as sexual age (the difference between the age at first sexual intercourse and current age) showed significant risk for the acquisition of HPV infection (Lenselink *et al.*, 2008). The sub-categories of this derived risk factors are < 11 years, 11 -19 years (these

are the high risk groups), 20-29 years and >30 years (these are low risk sub-categories). This risk factor was also derived in the study reported in this thesis.

2.5.1.3 Sexually Transmitted Infections

Epidemiological studies of sexually transmitted infections (STIs) other than HPV, with respect to the risk of cervical precursor lesions and cancer have demonstrated associations with *Chlamydia trachomatis* (CT), herpes simplex virus type 2 (HSV-2) and HIV (discussed as a immunocompromised risk factor), after controlling for strong risk factors such as HPV infection. A pooled analysis of multi-centre case-control studies conducted by the IARC provided evidence for significant associations of HSV-2 with SCC (odds ratio, OR= 2.2; 95% CI: 1.4–3.4) and ADC or ADSC (OR = 3.4; 95% CI: 1.5–7.7) in a group of HPV positive women. The association with *Chlamydia trachomatis* (CT) showed an increased risk for cervical cancer (OR = 1.8; 95% CI, 1.2–2.7). The association of cervical lesion with *Chlamydia trachomatis* has been determined based on both a history of infection (determined by antibody detection) and concurrent infection with HPV (Muñoz *et al.*, 2006; Samoff, 2005; Smith *et al.*, 2002a, 2002b, 2004). Concurrent CT infection has been shown as an independent risk factor (ORs of between 1.1 and 4.1) that promotes HPV infection (by inducing cervical microabrasions and inflammation) and HPV persistence (by immunological mechanisms) (Burchell *et al.*, 2006; Samoff, 2005). This formed the bases for using STI infection in this study as a surrogate indicator for a community at high risk for HPV infection and therefore the selection of the Lower Manya Krobo district for this study.

2.5.1.4 Inconsistent use of Condom

The promotion of the use of condom is one very important primary preventive method for STIs in general and more so for the prevention of HPV infection and cervical lesion (WHO;

Guideline Development Group, 2014; WHO; Chronic Diseases and Health Promotion Group, 2006). The consistent use of condom has been largely shown as a protective factor against HPV acquisition, promotes HPV clearance thereby reducing the risk of the development of cervical lesion and promoting regression of cervical pre-cancer lesion (WHO; Guideline Development Group, 2014; WHO; Chronic Diseases and Health Promotion Group, 2006; Shields *et al.*, 2004; Shepherd *et al.*, 2000).

Although it is the consistent use of condom that provides protection against HPV infection, its use provides partial protection since it does not protect against HPV infection that may be due to genital skin-to-skin contact or self-inoculation (Burchell *et al.*, 2006; Winer *et al.*, 2006). However, because it provides protection against other STI that promote the acquisition and persistence of HPV infection as well as the progression of pre-cancer lesions (section 2.5.1.3), it is very important to cervical cancer control and prevention. Therefore, it is important to determine the extent of the consistent use of condom during cervical screening as well as to determine factors that influence its consistent or inconsistent use. This will be useful in planning and targeting education on condom use and the need for consistent usage among a population. To this end, the study reported in this thesis determined the extent of condom use among women participating in the screening activity and analysed for the participant characteristics that may influence the inconsistent use of condom among the participants.

2.5.2 Reproductive Risk characteristics (Known Risk Factors)

2.5.2.1 *Multiparity*

The epidemiological data that support the role of parity (defined either as the number of full term pregnancies or number of live births) as a risk factor for cervical pre-cancer lesion and

cancer have mostly been from systematic/pooled reviews and meta-analysis of mostly case-control studies and a few large sample cohort studies, as was the case for most of these risk factors. Individual relatively small-sample studies (case-control or cross-sectional) are usually challenged by choice of controls, number of participants in subgroups and time of assessment of parity (at recruitment, during the study or at the end of follow-up) (Castellsagué and Muñoz, 2003a). Although parity is of a very low and non-significant risk for HPV acquisition and for low grade precursor lesions development, there are inconsistent reports of an association with HPV persistence and a moderate risk for progression of cervical lesions to *Carcinoma-in situ* (CIS) and invasive cervical cancer (Kim *et al.*, 2012; Vaccarella *et al.*, 2006). The two major sub-categories of parity are less than 3 full term pregnancies and 3 or more pregnancies. The latter (full term pregnancies of more than 3) are often significant high risk factors and mostly show a linear trend with the occurrence of CIS and invasive cancer. The 95% CI of OR of these association could range between 1.3 and 7.9 (Castellsagué and Muñoz, 2003a). Therefore, for this study, since the sample size was relative very small, determining the odds ratio of this association will be misleading. However, the distribution of the participants within the sub-categories of these characteristics were used as an indication of the likelihood of detecting cervical lesions among the women. This likelihood was thereafter compared with the detection of cervical lesion among the women by Pap test.

2.5.2.2 Oral Contraceptive Use

The current and duration of use of oral contraceptive (OC) as well as the duration since the stop of oral contraceptive use have consistently not been a significant risk for the acquisition of HPV infection. However, while the current and duration of use of oral contraceptive have been associated with an increased risk for the progression to high grade lesions and invasive

cervical cancer, the duration since the stop of oral contraceptive use has been associated with a reduced risk of these (Lenselink *et al.*, 2008; Vaccarella *et al.*, 2006). The two sub-categories for these risk factors are usage for less than 5 years and usage for between 5 and 10 years. Higher risks are observed among women who have been using oral contraceptives for 5-10 years (OR of between 1.4 and 5.4) and the highest risk was observed among users of more than 10 years (OR of between 2.0 and 7.8) (International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Castellsagué and Muñoz, 2003a; Smith *et al.*, 2003). Different study designs, and differences and difficulties in the choice of controls for case-control designs may influence the varying levels of risk as measured either by odds ratio or relative risk for cervical cancer in different studies (Shields *et al.*, 2004).

2.5.3 Other risk factors

2.5.3.1 *Smoking Status*

Current smoking status is associated with an increased risk of precursor lesions and invasive cervical cancer and as such is thought to play a role in HPV persistence/clearance. This risk has been low to moderate and inconsistent in most populations (Kapeu *et al.*, 2008; Lenselink *et al.*, 2008) and has been shown to be confounded by cofactors of sexual behaviour, such as the number of sexual partners (Vaccarella *et al.*, 2008). This confounding may come about because smoking is often associated with promiscuous life styles in most populations. Based on pooled analyses of both prospective and retrospective cohort studies, smoking generally is moderately associated with the risk of developing cervical cancer; OR range between 1.09 and 1.88 (Erickson *et al.*, 2013; Vaccarella *et al.*, 2008; Berrington de González *et al.*, 2004). However, compared to never smokers, current smokers are at the highest risk followed by ever smokers, while past smokers (of about 10 years) are often not-significantly at risk of cervical cancer as compared to never smokers (Sarian *et al.*, 2009). This is often explained on

the bases that during the period of smoking the related oncogenic agents accumulates in the body due to a slow clearance/excretions, However, following the stop of smoking, the level of the accumulated oncogenic agents reduces overtime to the levels as in non-smokers (Sarian *et al.*, 2009). The intensity of smoking of more than 15 or 20 cigarettes per day is often significantly associated with the risk for HPV prevalence and progression to cervical cancer, but the duration of smoking has not shown any such association (Erickson *et al.*, 2013; Vaccarella *et al.*, 2008; Berrington de González *et al.*, 2004).

2.5.3.2 *Immunological Status*

The two most important immunocompromised states, in relation to cervical cancer are HIV infection/AIDS and use of immunosuppressive medication, because they have been shown to be significantly associated with an increased risk of cervical HPV infection and progression of pre-cancer lesions (Erickson *et al.*, 2013; Firnhaber *et al.*, 2009; Veroux *et al.*, 2009). In a meta-analysis of the incidence of cancers among these peculiar populations, the risk of the incidence of HPV related cancers were increased, with HIV infected population bearing the highest risk for most of the cancers studied. Specifically for cervical cancer, the meta-analysis' standardized incidence ratio (SIR) range between 2.9 and 11.3 for HIV infected women and between 1.3 and 3.3 for organ transplant recipients undergoing immunosuppressive therapy (Grulich *et al.*, 2007). Issues of the interaction of HIV and HPV (including type specific concordance) are thought to account for the higher risk for the HIV/AIDS population although this is not clearly confirmed (Firnhaber *et al.*, 2009; Mbulawa *et al.*, 2009; Palefsky, 2009; Grulich *et al.*, 2007; Palefsky and Holly, 2003). Therefore, if a population has a large proportion of its members with such immunocompromised statuses, that population will be at a high risk of bearing a high burden of HPV infection and cervical pre-cancer lesion. The district that hosted the study (the Lower

Many Krobo district) described in this thesis has been reported to be one of the high burden of HIV infection in Ghana.

2.5.3.3 Inheritable Host Genetic Factors

Although this risk factor was not considered in this study, it is worth describing its contributions to cervical cancer. Based on limited data from a number of population based cohort studies, some variants of host inheritable genes have been suggested as possible risk factors for cervical cancer. For example, genetic variations in the *HLA DOBI* and *CD83* genes of the innate immune system have been shown to be associated with invasive cervical cancer in studies of families (Neuman *et al.*, 2000). Furthermore, homozygous alleles of single nucleotide polymorphism (SNP) at codon 72 of the *PT53* gene that changes the nucleotide C to G and a resultant replacement of arginine with proline in the p53 protein, have been suggested to lead to higher susceptibility to HPV-associated cervical cancer (Tommasino *et al.*, 2003; Storey *et al.*, 1998). This has been due to enhancement of the persistence of HPV infection and the progression of cervical lesions. Other such inheritable variations in genes such as, a C309G SNP in the MDM2 promoter, a C609T SNP in exon 6 of the NQO1 rs1800566 gene and another SNP C465T in exon 4 of the same NQO1, have been reported as associated with the development of cervical cancer (Hu *et al.*, 2010; Wang *et al.*, 2010; Wang and Hildesheim, 2003).

2.5.4 Summary of Risk Factors

The risk factors for the development of cervical cancer are mainly HPV infections and cervical pre-cancer lesions; however, some sexual and reproductive behaviour have been shown globally to consistently be risk factors for the acquisition of HPV infections, persistence of HPV infections, the development of cervical pre-cancer lesions and the

progression of cervical pre-cancer lesions to cervical cancer. However, it must be noted that of the two or more sub-categories of each of these risk factors, one or two are high risk while the others are low risk behaviours. For example age at first sexual intercourse has two sub-categories, < 20 years and ≥ 20 years and the former is high risk for the acquisition of HPV infection. The risk status of these sub-categories have been consistent globally but may be correctly determined or quantified with large size studies such as, multi-centre study, pooled-analysis or meta-analysis of studies. Therefore, this relative small-sample sized cross-sectional study will not be appropriate to quantify or confirm the risk status of these known risk factors. However, the distribution of the women between the high risk and low risk sub-categories will serve as a surrogate indicator of the risk status of the community.

2.6 THE HUMAN PAPILLOMAVIRUS

As discussed in the previous sections and indicated in figure 2.2, infections of HPV are the major determinant of the development of cervical cancer. As such, as part of this study, data on HPV infection was collected. It is therefore important to present a summary of the updated knowledge of HPV, in the content of this study.

2.6.1 Biologic Classification of HPV Types

In the study reported in this thesis, the detection of the genotypes and variants of HPV was a major objective that has implications for the selection of vaccines and HPV testing kit for cervical cancer screening in Ghana. As such, a brief description of the classification of the general group of viruses that HPV belongs is presented in this section. This group known as Papillomaviruses are a family of viruses referred to as *Papillomaviridae*, which is made of 29 genera at the most recent amendments (in 2004) (Bernard *et al.*, 2010; de Villiers and Gunst,

2009; de Villiers *et al.*, 2004). Of the 189 known types (more have been detected but not yet included in the classification), 120 have humans as host (these are referred to as human Papillomaviruses) while the others have other mammals, birds and reptiles as host (Bernard *et al.*, 2010; de Villiers *et al.*, 2004). The 120 human Papillomavirus (HPVs) have been grouped in 5 genera, these are Alphapapillomavirus, Betapapillomavirus, Gammapapillomavirus, Mupapillomavirus and Nupapillomavirus. These consist of 30 species (identified by numbers) that are further grouped as types (often referred to as genotypes) and subtypes (known as variants or lineage of a genotype). For example, the genus Alphapapillomavirus includes a species number-9 that consists of the following HPV genotypes 16, 31, 35, 33, 52, 58 and 67 (Bernard *et al.*, 2010) and the genotype HPV16 has variants HPV16Afr-1, HPV16Afr-2, HPV16AA1, HPV16AA2 and others. These classifications are based on the level of similarity/identity in the nucleotide sequences of the L1 gene of these viruses (the genome of the HPV is discussed in section 2.6.2 below). An HPV genotype is different from another if its full length genome and/or if the DNA sequence of its L1 gene differ by more than 10% of that of the other genotype. If this difference is between 1% and 10%, then the Papillomavirus is a subtype of the other genotype (as referred to as variant or lineage). If the difference is between 0.5% and 1%, then the Papillomavirus is of the same genotype as the other, but it is a sub-variant (also referred to as sub-lineage) of the genotype (Chen *et al.*, 2013; de Villiers and Gunst, 2009; de Villiers *et al.*, 2004). In the study reported in this thesis, the genotypes of the detected HPV were determined and variants of the HPV genotypes 16, 18 and 45 were determined. These will help understand how different the variants in Ghana may be from the known variants of these genotypes and understand the implication of these data on the selection of an HPV testing kit for HPV screening in Ghana. Additionally, these may contribute to studying the evolutionary trends of these genotypes in Ghana and the association of HPV variants and cervical lesions and cancer development.

2.6.2 The HPV Genome

As indicated in the previous section the classification or identity of an HPV depends on the whole genome or the L1 gene sequence and in furtherance of this understanding, this section provides a description of the genome of the generalized HPV. The human Papillomavirus particle is made-up of a circular double stranded 7.9 kbp DNA genome encapsulated by a two protein shell of size 50 nm. The genome consists of nine open reading frames (ORF) that control eight genes (Figure 2.3). Six of these genes are transcribed during the early stage of the life cycle of the virus and therefore are referred to as the early genes; these are *E1*, *E2*, *E3*, *E4*, *E5*, *E6*, *E7* and *E8*). The others are transcribed during the late stages of the life cycle of the HPV and so are referred to as late genes (*L1* and *L2*). The ninth ORF is the long control region (LCR) also referred to as the upstream regulatory region (URR). It controls the expression of the whole genome (Alp Avcı, 2012; Fertey *et al.*, 2010; de Villiers and Gunst, 2009) and therefore variation in its sequence has implication for oncogenicity of the detected HPV variant. For this purpose as well as for variant classification, the study reported in this thesis determined the variation in the LCR of 3 selected genotypes.

The other genes of the HPV genome have been shown to have the following function. Three of the early genes *E5*, *E6*, and *E7*, are oncogenes that negatively influence the host cells' control of transcription and cell cycle, thereby inducing the transformation to malignancy. It should however be noted that the Beta-papillomavirus and the Gamma-papillomavirus do not have the *E5 gene* and therefore do not encode the E5 protein shell (Venuti *et al.*, 2011; Muñoz *et al.*, 2006; de Villiers *et al.*, 2004).

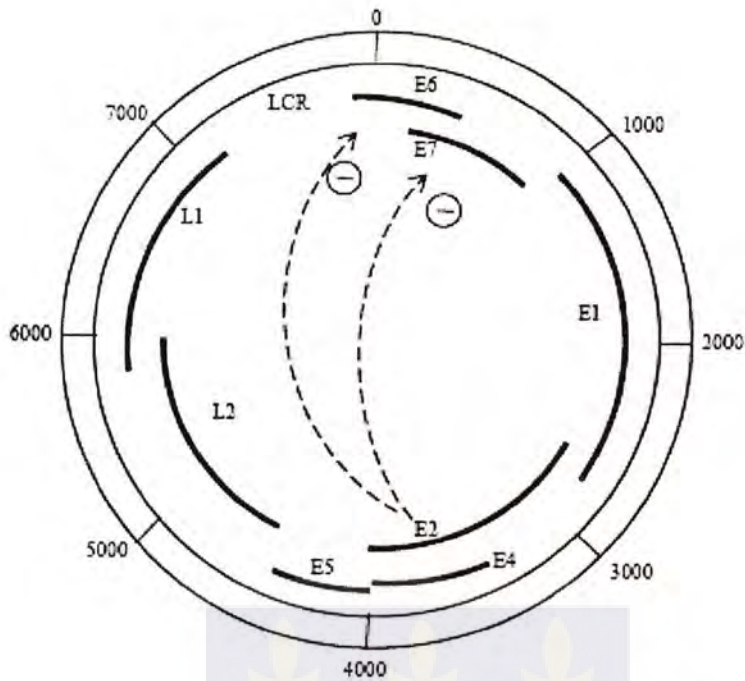


Figure 2. 3: An illustration of the double stranded DNA genome of HPV.

Adopted with modifications from Villa, 2006; Soltar *et al.*, 2004; Lukaszuk *et al.*, 2003

The other two, *E1* and *E2*, are involved with the regulation of the transcription and replication of the viral genome. The *E2* protein (a product of the *E2* gene) also suppresses the expression of the *E6* gene when the viral genome is not integrated in the host genome. Additionally, the product of the *E8* gene, which is expressed as a fusion protein with the C-terminal of the *E2* protein, known as E8^{E2C} (a protein known only to be expressed by HPV genotypes 1, 11, 16, 31, and 33), in addition to the *E2* protein repress the *E6/E7* promoter, resulting in controlling the expression of the *E6/E7* oncogenes and is also involved in the control of viral replication (Dreer *et al.*, 2016; Straub *et al.*, 2015, 2014; Fertey *et al.*, 2010). The function of the recently identified small putative *E3* gene are yet to be identified and no protein expressed from it has also been identified (Alp Avcı, 2012). The two late genes, *L1* and *L2*, code for the structural proteins that compose the viral capsid/shell (Muñoz *et al.*,

2006; de Villiers *et al.*, 2004). It is worth noting that the available vaccines for HPV are viral-like-proteins (VLP) based on the products of the *L1* and *L2*.

Although classified as an early gene, the E4 gene product has been suggested to play roles in the late stage of the HPV life cycle and these roles have been a subject of speculation as it has remained largely unclear (Doorbar, 2013). The suggested major role of the E4 protein has been its involvement with virus release after assembly, due to its association with cytokeratin network reorganization (Doorbar, 2013). Additional suggested roles for the E4 protein have been in HPV genome amplification, expression of capsid proteins, and possible perturbations in RNA processing, signal transduction, protein degradation or cell differentiation (Doorbar, 2013; Peh *et al.*, 2004).

2.6.3 Summary of the HPV

The HPV is one of a number of Papillomaviruses that is made up of several genera, species, genotypes and variants of each genotype; the classification of which is based on DNA sequence variations of some of the genes of its 7.9 kbp circular genome. The function of the products of its nine open reading frames synergistically defines its oncogenicity and the variation therein with different genotypes. Implying that based on the distribution of the different genotypes in a population, the risk of cervical lesion development will vary.

2.7 THE NATURAL HISTORY OF HPV INFECTION

The natural history describes the role of HPV in the development of cervical cancer, as has been mentioned in the previous sections. It is the understanding of the natural history that has informed the development of screening methods for cervical cancer and has greatly supported cervical cancer related research. The study reported in this thesis was largely informed and

designed to assess data representing most of the stages in the natural history. Therefore, an understanding of the description of this section is critical to the understanding of this study.

2.7.1 Association of HPV with Cervical Cancer

The causal relationship between HPV and cervical cancer was indirectly established through the findings of epidemiological studies that initially identified some sexual behaviours as significant risk factors for cervical cancer. It was so until the employment of molecular techniques in epidemiological studies (molecular epidemiology) that it became undoubtedly clear that the sexual behaviour risk factors were only indicative of the risk of HPV infection and that it was persistent HPV infection which was a risk factor for the development of cervical pre-cancer lesions (Bosch *et al.*, 2002; Franco *et al.*, 1999; Walboomers *et al.*, 1999). Furthermore, reviews and pooled multi-centre studies have shown that between 90% and 99% of cervical cancer cases in most world regions were associated with HPV infections and odds ratios (ORs) of between 35 and 130 have been reported for these associations (Muñoz *et al.*, 2003; Bosch *et al.*, 2002). Using the causality criteria for human diseases, Bosch *et al.*, (2002), have discussed in detail the causal relationship between specific genotypes of HPV and cervical cancer. That report indicated that of the over 170 HPV characterised types, only about 40 infect the anogenital area, leading possibly to the development of cervical pre-cancer lesions and eventually cancer. These 40 types are often referred to as genital HPV. These imply that, however few, there are some cervical cancers that may not be due to persistent HPV infections and so studies may report cervical pre-cancer lesions among women who are HPV negative.

The major processes in the natural history of HPV infection (acquisition, clearance, persistence and progression) are considered as a spectrum of occurrences in association with

the multi-step natural history of cervical carcinogenesis. These are illustrated in figure 2.4 below.

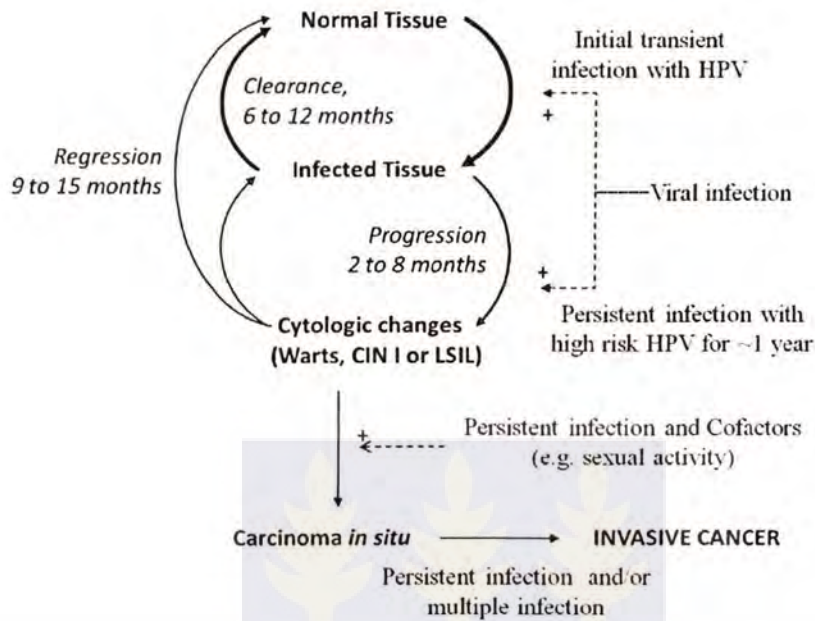


Figure 2. 4 An illustration of the natural histories of HPV infection and cervical cancer.

Adopted and modified based on data from (Goldstein *et al.*, 2009; Kahn, 2009; Schiffman and Wacholder, 2009; Flores *et al.*, 2006; Schiffman and Castle, 2005). The relative thickness of the arrows indicates the proportion of cases that progress from one state to the next. Clearance is immunologically mediated.

2.7.2 Acquisition of HPV Infection

Human Papillomaviruses are ubiquitous and the commonest of sexually transmitted infections (STI), particularly common among young sexually active women. Although it infects the cutaneous and mucosal epithelia of most parts of the body, its infection of the metaplastic epithelium of the cervical transformation zone is of greater clinical significance (Lenselink *et al.*, 2008; Moscicki *et al.*, 2006). It is clear that almost all forms of sexual contacts, including penetrative intercourse, genital contacts and genital skin to skin contact

with an infected partner is necessary for the transmission of the virus. In addition, self-inoculation also aids in the spread of the virus to other body locations, most likely by skin to skin contact (Moscicki *et al.*, 2006; Schiffman and Kjaer, 2003). Other known routes of transmission, including, vertical and perinatal transmission, and trans-placental (intrauterine) transmission, have very marginal implication for the development of cervical cancer (Bosch *et al.*, 2006).

2.7.3 Persistence and clearance of HPV Infection

The figure 2.3 indicates that acquiring an HPV infection does not mean a woman may develop cervical pre-cancer lesion, since it is the persistence of the HPV infection that is required for the development. However, some persistent HPV infections may eventually be cleared even before any cervical pre-cancer lesion develops (Goldstein *et al.*, 2009; Kahn, 2009; Schiffman and Wacholder, 2009; Flores *et al.*, 2006; Schiffman and Castle, 2005). Therefore, it is possible for a woman to be HPV positive and not have any cervical pre-cancer lesion. Persistent HPV infection is loosely described as the detection of the same HPV type two or more times within a period of testing (there are no standard or recommended periods), otherwise that infection is said to have been cleared (Koshiol *et al.*, 2008; Moscicki *et al.*, 2006; Schiffman and Kjaer, 2003). Different time intervals between testing have been reported by studies resulting in variations in the definition of HPV persistence/clearance; while some study have reported 6 to 12 months duration, others have reported long periods of between 5 years and 7 years (Koshiol *et al.*, 2008; Moscicki *et al.*, 2006; Schiffman and Kjaer, 2003). Inherently, there are no recommended durations indicated neither for research nor as part of cervical cancer prevention programme guideline. It is worth noting that differences in HPV type-specific clearance and persistence rates do exist, such that some HPV genotype are easily cleared than others. One of such order of clearance for high risk

HPV infection (from highest to lowest) was reported as follows, HPV- 59, 35, 45, 18, 33, 52, 31, 58 and 16, while that of persistence was HPV 52, 16, 58, 33, 31, 35, 45, 18 and 59 (Insinga *et al.*, 2011).

2.7.4 Progression of HPV Infection

The important characteristics that influence the ability of an HPV to lead to pre-cancer lesions and to cervical cancer are primarily, the genotype of the infecting HPV, the length of persistence of the HPV infection, host characteristics and exposure to some risk factors (as discussed in section 2.5) (Insinga *et al.*, 2011; Moscicki *et al.*, 2006; Schiffman and Kjaer, 2003). These imply that cervical pre-cancer lesions (cytological changes) in themselves are risk factors for cervical cancer since some of the pre-cancer lesion may regress (figure 2.4).

A period of between 10 years and 25 years elapses before a persistent HPV infection may finally result in the development of cervical cancer (Bosch *et al.*, 2008; McCredie *et al.*, 2008; Woodman *et al.*, 2007; Moscicki *et al.*, 2006; Schiffman and Kjaer, 2003). This provides a long enough time for the detection, and treatment of cervical pre-cancer lesions and therefore, enough time for the prevention of the cancer that may develop if the pre-cancer lesions are not detected and treated.

2.7.5 Molecular Mechanisms of HPV Mediated Carcinogenesis

The roles of the genes of the HPV genome and their relationship with the natural history of cervical cancer (discussed earlier) together contributes to the understanding of the mechanism of HPV mediated carcinogenesis. Although the related genotype specific HPV biological mechanism of the carcinogenesis is not completely and clearly understood, this section presents a generalized model of the carcinogenesis of HPVs. This is particularly true of a group of HPV genotypes referred to as high risk HPV types (HR HPV). As will be discussed

in sub-section 2.8.1, these are more effective at causing the progression of pre-cancer lesions to cancer (Wang and Hildesheim, 2003).

The principal genes responsible for oncogenicity are the *E6* and *E7* oncogenes, as well as the *E2* gene, if the viral genome is not integrated (Venuti *et al.*, 2011; Lowy *et al.*, 2008; Muñoz *et al.*, 2006). The oncogenicity of the *E6* and *E7* proteins of each HPV genotype has to do primarily with the strength of their interaction with a number of cellular proteins, however only few of these are understood (Venuti *et al.*, 2011; Lowy *et al.*, 2008; Muñoz *et al.*, 2006). The most understood of these interactions are those between the oncoproteins (*E7* and *E6*) and two tumour suppressive proteins, retinoblastoma protein (pRb) and p53 protein respectively. The interaction between the HPV *E7* protein and human pRb protein results in an increased DNA replication in the infected cell and therefore unscheduled (uncontrolled) cell-cycle activities occur. Such cells are expected to be lead to cell death (be killed) through a process mediated by the actions of the protein p53. However, the HPV *E6* protein binds with this p53 protein, and leads to the degradation of the p53. As a result, there is a loss of cell cycle control, and these cells usually are not killed. These result in an increased loss of cell-cycle control, and genomic instability, which may eventually lead to cell immortalization and malignant transformation (Venuti *et al.*, 2011; Lowy *et al.*, 2008; Muñoz *et al.*, 2006). For a viral genome that is not integrated in the host genome, the *E2* viral protein negatively influences the transcription (formation) of the *E6* and *E7* viral proteins, (reduces the amounts produced). However, when the viral genome integrates into the host genome, the integration occurs at the *E2* gene of the virus, thereby disrupting the formation of *E2* protein and related function. This ensures that the *E6* and *E7* proteins are produced at a faster rate, with the ultimate result of cancer development (Lowy *et al.*, 2008; Muñoz *et al.*, 2006). It is therefore understandable that HPV genotypes that are able to integrate their genome more often tend to be more efficient at tumour progression. The role of another gene product, the

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E5 protein, is still not clearly understood; however, it has been shown to be a weak transforming protein *in vitro*. It interacts with a number of cellular proteins involved with HPV life cycle, cell transformation, cell proliferation and evasion of the immune response (Greco *et al.*, 2011; Venuti *et al.*, 2011; Genther *et al.*, 2003). For example HPV16 E5 protein has been shown to interact with and down-regulate the Major Histocompatibility Complex (MHC) class I and class II leading to the evasion of immunosurveillance of the immune system. It interacts and activates the epithelial growth factor receptor (EGF-R) involved with cell proliferation and also interacts with the HPV E6 oncoprotein to enhance the oncogenic activities of both proteins (Greco *et al.*, 2011; Venuti *et al.*, 2011). The HPV16 E5 has also been shown to be able to down-regulate the expression of the tumour suppressor proteins p21 and p27 (Venuti *et al.*, 2011). Therefore, a high prevalence of HR HPV infection increases the risk for the development of pre-cancer lesions and cervical cancer. Additionally, and although not part of the study reported in this thesis, sequence variation within these genes (*E5*, *E6*, *E7*) may have implication for variation of the risk of developing pre-cancer lesion within a population.

2.7.6 Summary of the Natural History of HPV infection

The natural history indicates that HPV infections are necessary for the development of cervical pre-cancer lesion and therefore cervical cancer, however, most HPV infection do not lead to cervical pre-cancer lesions because the infection are often cleared by human immune system. It is the few HPV infections that persist that are able to induce the development of cervical pre-cancer lesion. Furthermore, the cervical pre-cancer lesions that may develop may also progress to cervical cancer or may regress. The small proportion of women who may develop cervical pre-cancer lesions and cervical cancer do so as a result of the additional continuous exposure to risk factors, most of which are the sexual and reproductive behaviours

of these women. The long duration between acquiring and maintaining an HPV infection, and the development of cervical cancer (10-25 years) provide a long enough window of opportunity to prevent the development of cervical cancer.

2.8 EPIDEMIOLOGY OF HPV INFECTION

Data generated by the study reported in this thesis was primarily focused on providing the country-specific HPV molecular epidemiological information for a community in Ghana. This section provides the necessary background information at the Global, African and Ghanaian levels so as to put in context the contribution of this study to knowledge on HPV in Ghana. This will include a description of the risk types of HPV, prevalences of HPV stratified by grade of lesion, age and country.

2.8.1 Epidemiological Risk Types Classification of HPV

As indicated in section 2.7.6, each HPV genotype's (the over 40 genital HPVs) ability to cause cervical cancer (also referred to as oncogenic potential/risk) differs and this has formed the basis for a classification of HPV into risk type categories. High risk HPV types are those that are most prevalent, most associated with high grade and invasive cervical cancer and often occur as single infections in high grade lesion (Muñoz *et al.*, 2003). This molecular epidemiologic-based classification, categorises the following types as high risk HPV types (HR HPV); HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82. The following; 6, 11, 40, 42, 43, 44, 54, 55, 57, 61, 70, 71, 72, 81, 84 and CP6108 have been categorized as low risk HPV types. The HPV genotypes 26, 30, 34, 53, 66, 67, 69, 85 and 97 are considered as probable high risk while HPV types 34, 57, and 83 were considered to be associated with undetermined risk (Muñoz *et al.*, 2003). Therefore, the prevalence or distribution of HR HPV in a community will be indicative of a high risk for cervical pre-cancer development. For the

study reported in this thesis these determinations were made. The following sections describe the different prevalences of HPV infections that are important for a population.

2.8.2 Prevalence of HPV Infection

Among every general population, the overall HPV prevalence, HPV genotypes-specific prevalence, HPV risk type prevalences and age-specific HPV prevalences are important information for cervical cancer prevention. These have been shown to vary with the grade of cervical lesion (normal, low grade and high grade) and therefore stratified HPV infection by grade of lesion are importance information (Bruni *et al.*, 2010; de Sanjosé *et al.*, 2007). Also varying are geographical distributions of HPV prevalences both within and between countries. Different HPV prevalences have also been reported among some peculiar populations, defined by sexual practices or immunocompromised state, for example commercial sex workers and HIV infected persons respectively (Ortiz *et al.*, 2006). Therefore, the proportion of such peculiar population among the study population is an important information to note. Due to these variations, it always important for every country to determine its county-level data to inform its planning of a cervical cancer prevention programme (WHO; Guideline Development Group, 2014); which was one of the major objectives of the study reported in this thesis.

It must be noted that in addition to the geographical and biological factors that contribute to the observed variations in HPV prevalences, issues such as study design (case-control, cross sectional etc.), the methods of HPV detection and genotyping primers used may also contribute marginally and often none significantly to the observed variations (de Sanjosé *et al.*, 2007; Ortiz *et al.*, 2006; Clifford *et al.*, 2005a).

2.8.2.1 *HPV prevalence variations with grade of lesion and age*

The global distributions of HPV infection among women with normal cytology have indicated consistently that the African region has a high HPV prevalence. According to the report of a multi-centre study, Europe recorded the lowest average prevalence of 5.2%. South America and Asia regions recorded, 14.3% and 8.7 % respectively, while sub-Saharan Africa recorded the highest prevalence of 25.6%; it is worth noting that the pooled analysis included only one study from sub-Saharan Africa and as such that data may be biased by an under representation (Clifford *et al.*, 2005b). In a more current systematic analysis (de Sanjosé *et al.*, 2007) that included more countries for each world region (78 studies in all), a similar trend was observed with much more detail. The highest overall HPV prevalences among women with normal cytology were observed in the following five world regions, three of which are in Africa; Eastern Africa (31.6%), Eastern Europe (29.1%), Northern Africa (21.5%), Central America (20.4%) and Western Africa (17.0%). The five world regions with the lowest overall HPV prevalence did not include any region of Africa, these were Southeast Asia (6.2%), Southern Europe (6.8%), Japan/Taiwan region (7.0%), South-Central Asia (7.5%) and Northern Europe (7.9%). These imply that there was a high possibility of recording a HPV prevalence in the study reported in this thesis, since it was conducted in an African community, as has been observed.

Among women with low grade cervical precursor lesions, the overall HPV prevalence, as reported in a meta-analysis of 55 studies (8,308 women) from different geographical regions (Clifford *et al.*, 2005b), also showed variations, but with higher HPV prevalences. North America recorded the highest prevalence of 80.1%, followed by South/Central America, 68.3%; Europe, 67.8%; Asia, 67.1% and Africa 59.1%. However, the prevalence of HPV infections in high grade lesions and invasive cervical cancer shows very little variation but

were very high (70% - 99%). In a meta-analysis of studies from different world regions, an overall prevalence of between 84.2% and 87.6% was reported with less variation across geographical areas of the world (Clifford *et al.*, 2003).

2.8.2.2 Age-specific HPV prevalence variations

The age-specific distributions of overall HPV infections among women within the general population show an overall trend that is largely similar in most world regions. For example, in a meta-analysis of 78 multi-centre-study of women with normal cytology, it was observed that women younger than 25 years were most infected with HPV (with a peak between 20 - 22 years). This was followed by a progressive decrease in prevalence with age until a small increase was observed in women between 34 years and 54 years (Lenselink *et al.*, 2008; de Sanjosé *et al.*, 2007; Burchell *et al.*, 2006; Schiffman and Castle, 2005). Despite, general trend, there were major variations among the different geographical areas and mainly with the age range for which a second increase in prevalence occurred; for Africa and Europe the age range of 45-54 years experienced a small second increase in prevalence, while for the North, Central and South America regions, the second increase in prevalence was among the 34-45 years range. Data from the Asia region did not show a second increase in prevalence as observed for the other regions (de Sanjosé *et al.*, 2007).

Furthermore, age-specific HPV prevalence variations have been observed at the country level. For example, analysing data of women with normal cytology, it was observed that data from Thailand and Vietnam showed two peaks at 25-34 years and 55-64 years, data from Columbia, Argentina and Chile all showed one peak at 25-34 years, 35-44 years and 35-44 years respectively (Clifford *et al.*, 2005a). Data from Italy and Netherlands showed one peak at 55-64 years and 45-54 years while data on women in the general population in Spain

showed two peaks at 20-24 years and 45-54 (Bosch *et al.*, 2006). Data were only available from a Nigerian study for Africa and this showed two peaks, at 25-34 years and 45-54 years (Clifford *et al.*, 2005a). Another study among women in the general population in Canada, which detected only high risk HPV types, showed a single peak at 20-24 years (Ogilvie *et al.*, 2013). Therefore, it is important for every country to generate its own data to be able to understand the dynamic of HPV prevalence among its population in order for the effective planning of an HPV and cervical cancer prevention programme. The study presented in this thesis provides such data for a community in Ghana using two separate cervicovaginal specimens from each woman.

2.8.2.3 *Type specific HPV prevalence*

As indicated in section 2.8.1, high risk HPV are most commonly detected in cervical cancer tissues and as such the high risk genotypes HPV 16 and 18 are the most prevalent HPV types among women globally; although there are variations in the HPV genotype specific prevalence among women with normal cytology in the different world regions (de Sanjose *et al.*, 2007). Specifically, a pooled analysis reported by de Sanjose *et al.*, (2007) showed that among women with normal cytology, HPV 16 was the commonest genotype in all regions except in Eastern Africa and Japan/Taiwan regions where HPV 52 was the commonest. However, it must be noted that the data on HPV prevalence in Africa included in that pooled analysis was very limited and therefore the reported type specific prevalence was most likely not a true reflection of the situation in Africa. This limitation of data from Africa was one of the motivations for the conduct of the study reported in this thesis, which was aimed at providing this and other importance information on HPV prevalence.

Furthermore, even wider variations were observed when the second commonest HPV types in each of the regions were considered; Eastern Africa and Japan/Taiwan region (HPV 16); Eastern Europe, Central America (HPV 31); Northern Africa, Northern Europe, Western Europe, Eastern Asia, Southeast Asia (HPV 18); and Western Africa and South America (HPV 58); North America (HPV 53), Southern Europe (HPV 66) and South-Central Asia (HPV 42). These variations confirmed the observation of an earlier review of studies among women with normal cytology by Clifford *et al.*, (2005b), which reported the following as the three most common HPV types in each of these regions; Sub-Saharan Africa (HPV 42, 16 and 35), Asia (HPV 16, 42 and 33), South America (HPV 16, 58, and 18); Europe (HPV 16, 31, and 18).

These variations in HPV type specific prevalence are also observed among women with low grade lesions, but to lesser extent (Clifford *et al.*, 2005a). The three most prevalent HPV types observed for each of the regions were as follows, North America (HPV 16, 51, 56), Europe (HPV 16, 31, 51), Africa (HPV 16, 53, and 58), South/Central America (HPV 16, 33 and 6) and Asia (HPV 16, 58, and 18). A further narrower variation in HPV prevalence was observed among women with high grade and invasive cervical cancer, HPV 16, 18, 33, 31 and 45 were the commonest with HPV 16 and 18 accounting for 66.9% of all HPV infection in all world regions (Clifford *et al.*, 2005b).

It must be stated that these reported prevalences of the genital HPVs were both as single and multiple type infections; however, higher rates of single infections were often observed. These multiple type infections were most often observed among younger women and women with immunosuppressive conditions. They were often also observed among women with lower grade lesions than high-grade lesion and normal cytology (Trottier, 2006). Although different combinations of genotype multiple infections have been reported, some studies have

generated data that seem to suggest some level of association between specific HPV types that are within the same species (Mejlhede *et al.*, 2010; Conesa-Zamora *et al.*, 2009; Awua *et al.*, 2007). Also, the limited data on whether HPV genotypes compete or facilitate another HPV genotype's acquisition or persistence makes it difficult to understand clearly the influence of multiple infection on HPV prevalence and therefore how HPV prevalence might change in this era of type specific vaccination (Rositch *et al.*, 2011).

2.8.2.4 Country specific HPV prevalence

Another fact that encourages the determination of country-specific data on HPV infection was the well identified inter- and intra-country levels variation. HPV studies in Spain serves a good example of this intra-country variation. Specifically, studies in Madrid and Murcia have reported HPV prevalence of 20.2% and 49.7% respectively among women attending cervical pathology clinics (Martín *et al.*, 2011; Conesa-Zamora *et al.*, 2009). These studies also reported HPV genotypes 16, 53, 31, 52 and 6 as the commonest types in Madrid (Martin *et al.*, 2011) and HPV 16, 45, 51, 33 and 31 as the most detected in Murcia (Conesa-Zamora *et al.*, 2009). Furthermore, these studies have discussed how similar and different their data were relative to studies in other countries in Europe such as France and Italy (Martín *et al.*, 2011; Conesa-Zamora *et al.*, 2009).

The inter-country variations in HPV prevalence is exemplified by the few studies from the West Africa region; a community-based study in Ibadan, Nigeria determined that the 95% confidence interval of overall HPV prevalence was between 24.8% and 26.3% among women with normal cytology. HPV 42, 16, 35, 31 and 81 were the five most prevalent HPV genotypes reported by that study (Thomas *et al.*, 2004). However, a hospital based study in Benin, reported a 95% confidence interval for the overall prevalence as between 26.7% and

33.2% among women with normal cytology; HPV 59, 35, 16, 18, and 58 were detected the most (Piras *et al.*, 2011). Furthermore, a study in the Gambia determined an overall prevalence of 13.4% and HPV 16, 35, 18, 33 and 58 as the most prevalent HPVs (Wall *et al.*, 2005). In yet another study of women 35 years or older in Senegal, an overall prevalence of 18.0% was reported and HPV 16, 58, 18, 33 and 54 were the commonest HPV types (Xi *et al.*, 2003). A hospital based study in Accra, Ghana that did not report type specific prevalence, reported an overall prevalence of 10.7% (Domfeh *et al.*, 2008). In a case-control study in Abidjan, Côte d'Ivoire, HPV prevalence among the control group (non HIV positive) were 31.1%; the overall most common HPV types was confounded by the HIV positive status of the cases and therefore is not considered herein (Adjorlolo-Johnson *et al.*, 2010).

In respect of the prevalence of HPV infection among women diagnosed with advance cervical cancer both inter- and intra-country variations were very minimal and often negligible. For instance, Two studies of women diagnosed with cervical cancer in Ghana recorded HPV prevalences of 89.8% (95% CI 85.7-93.4) and 98.0% (95% CI 92.6-99.8) with the following as the most prevalent, HPV 18, 59, and 45 in both studies (Awua *et al.*, 2016; Attoh *et al.*, 2010; Awua *et al.*, 2007). Similar prevalences were reported by multicentre study in Ghana, Nigeria and South Africa (Denny *et al.*, 2014).

2.8.3 Summary of Epidemiology of HPV

The body of knowledge on the epidemiology of HPV indicates a lot of variability. Genotypes of HPV have shown differences in their risk potential for inducing cervical pre-cancer lesions and therefore cancer. The extensive variations in prevalence have been seen in respects of their distributions among the different grades of cervical pre-cancer lesions and cervical cancers, such that there is high variability among low grade lesions but lower

variability among high grade lesions and cancer. This extensive variability is also seen in the age-specific, country level genotype specific prevalences.

2.9 PREVENTION OF CERVICAL CANCER

The primary aim of the study reported in this thesis was to provide information that was needed for the planning of an effective cervical cancer prevention programme in Ghana; as such, this brief description of the prevention of cervical cancer was necessary as a background to the study. Although there are primary, secondary and tertiary prevention of cervical cancer, this description is limited to only primary and secondary prevention, based on the context of this study.

2.9.1 Primary Prevention

Primary prevention refers to taking measures to ensure that a disease does not occur among individuals at risk by ensuring minimal or no exposure to the associated risk (Shepherd *et al.*, 2000). For cervical cancer, this will include measures to prevent acquiring and maintaining persistent HPV infection. Until the mid-2000s, the major primary prevention of cervical cancer had been the use of educational intervention, aimed at promoting sexual-risk reduction behaviours. These included abstinence from sex before marriage (casual sex), delay of first sexual intercourse, reduction of the number of sexual partners and consistent use of condoms (Shepherd *et al.*, 2000). However, following the association of HPV with cervical cancer and the subsequent development of the HPV vaccines, prophylactic vaccination against two, four or nine of the about 40 genital HPV have become the major promoted primary prevention strategy for prevention of cervical cancer.

2.9.1.1 Preventive Sexual Behaviour

As with most STI, the use of preventive education on abstinence does not seem effective and may not have any significant effect in the prevention of cervical cancer, both globally and in Ghana. For example, in spite of the numerous sexual educational campaigns in the Damgbé West district, in Ghana, the incidence of teenage sexual activity, which is a surrogate for abstinence, has been estimated to still be high; a study in that district (close to the location of the study reported in this thesis) reported that 55% of teenagers were sexually active by the age of between 13 years and 19 years (Afenyadu and Goparaju, 2003). Similarly, reports in other countries have indicated that educational campaigns encouraging young women to abstain from sexual activities till marriage or/and reduce their number of sexual partners, did not seem to have had any noticeable effect on the incidence of HPV infection, cervical lesions and cervical cancer (Kahn, 2009). These sexual risk reducing behaviour interventions have been observed to be effective for up to 6 months, and showed different levels of behavioural changes. This low duration of changes in behaviour will most likely not be adequate to influence a reduction in the incidence of cervical cancer (Norton *et al.*, 2012; Picot *et al.*, 2012; Shepherd *et al.*, 2000).

Consistent use of condom by male partners was reported to be associated with a reduced risk for HPV infection and cervical lesion. In a study by Winer *et al.* (2006), it was observed that compared to women whose male partners use condom at a frequency of 5% or less, women whose male partners use condom 100% of the time during penetrative sexual contact were 70% less likely to be infected with HPV. Furthermore, women whose male partners used condom for more than 50% but less 100% of the time were 50% less likely to be infected with HPV. Also, although not significant, the use of male condom was associated with a less likelihood of developing cervical lesions by female partners. The fact that HPV transmission

is possible by genital skin to skin contact and the male condom does not cover the peno-scrotal region explained the less than 100% protection among women whose male partners used condom always (100%). The study reported in this thesis provided community education on sexual risk behaviours associated with cervical cancer development

2.9.1.2 Prophylactic Vaccination

This section describes the development and the current state of efforts at promoting HPV vaccination in the prevention of cervical cancer. Following the findings that persistent infection with high risk HPV is the causal factor of cervical cancer, one of the major developments in cervical cancer prevention has been the production of three prophylactic vaccines; these are Gardasil (which protects against infections of HPV16, HPV18, HPV6 and HPV11), Cervarix (which protects against HPV16 and HPV 18) and a nonavalent vaccine (which protect against HPV6, 11, 16, 18, 31, 33, 35, 45, and 58) (Chatterjee, 2014; Van de Velde *et al.*, 2012; PATH; Practical experience, 2010a; Dunne *et al.*, 2008).

The first WHO position paper on HPV vaccination encouraged a programmatic approach to a national vaccination scheme that selects the target population based on the population's data on the age of initiation of sexual activity. In addition, the cost effectiveness of the HPV vaccination strategies as well as sustainable funding should be considered before implementation. As such, the target group for cost-effective vaccination in most countries were reported to be adolescent females ranging between the ages of 9/10 years and 13 years (PATH; Practical experience, 2010b; Kim and Goldie, 2008). On the other hand, vaccinations in boys have been considered by some studies as not cost-effective, if a good coverage were achieved for girls, and so vaccination for boys has mostly been uncommon (Kahn, 2009; Barnabas *et al.*, 2006).

In order for developing countries (most affected by HPV infection and related diseases) to overcome the challenges of cost-effectiveness and sustainable funding, a number of initiatives and demonstration programmes have actively contributed to the reduction in the cost of HPV vaccine for developing countries. Initiatives such as the PATH HPV Vaccination Pilots, Gardasil Access Programmes and Global Alliance for Vaccines and Immunisations (GAVI) initiatives have been undertaken in countries such as India, Peru, Uganda and Vietnam (Banura *et al.*, 2012; Gulland, 2012; Ramanathan and Varghese, 2010). African countries such as Tanzania have already benefited, while since February 2013, countries such as Ghana, Kenya, Madagascar, Malawi, Niger, and Sierra Leone have been participating in GAVI supported demonstration programmes (GAVI Alliance Support, 2013; Quentin *et al.*, 2012; Watson-Jones *et al.*, 2012). In spite of the fact that routine HPV vaccination in Ghana has been shown to be an appropriate option, and that an HPV vaccines pilot has started in Ghana, the need for secondary prevention (screening) remains relevant and must be supported with relevant empirical data on baseline HPV prevalence by studies such as that reported in this thesis (Edwin, 2010).

2.9.2 Secondary Prevention: Cervical Screening

The study reported in this thesis is primarily a secondary prevention activity that employed two of the three known cervical screening methods. This section provides a description of the development of the three available cervical screening methods (cytology, visual inspection and HPV testing), the advantages and limitations, as well as the bases of the choice of which to use in this or any other study.

As has been described thus far, once a woman is persistently infected with HPV or has developed a cervical pre-cancer lesion (whichever is detected first), there is a risk for cervical cancer development and thus the need to detect and stop the progression of the pre-cancer

lesion. Despite the fact that about 90% of HPV infections are often cleared and lesions may regress in about 1 or 2 years (figure 2.4), the fact that the burden of cervical cancer is still high, calls for action to reduce the burden. Measures taken to achieve these (detection of HPV and cervical pre-cancer lesions by screening and the treatments of detected precursor lesions) constitute secondary prevention of cervical cancer. Consistent cervical screening and appropriate treatment of pre-cancers have so far been the most effective, at reducing cases and deaths attributable to cervical cancer (Sanghvi *et al.*, 2008; Sankaranarayanan *et al.*, 2005).

Of the three well studied screening methods for the early detection of pre-cancer lesions, cytology (Papanicolaou or Pap smear) has been the most effective (Sanghvi *et al.*, 2008; Sankaranarayanan *et al.*, 2005). However, visual inspection of the cervix after the application of either acetic acid (VIA) or Lugol's Iodine (VILI) has been useful in the detection of pre-cancer lesions in developing countries (Sanghvi *et al.*, 2008; Sankaranarayanan *et al.*, 2005). The third approach, which involves the detection of HPV DNA (HPV testing), has shown great promise, however, some few challenges remain to be addressed in order for it to be implemented as a primary testing method in routine clinical practice of most countries (Cuzick *et al.*, 2012).

Screening activities remain relevant even with the development of vaccines against HPV infection, because while a small fraction of cervical cancer are not due to HPV infection (Clifford *et al.*, 2003), not all high risk HPV infection are protected against by HPV vaccines available (Chatterjee, 2014; Van de Velde *et al.*, 2012; PATH; Practical experience, 2010a; Dunne *et al.*, 2008). Additionally, for monitoring the progress and effectiveness of vaccination at protecting cervical cancer, there is the critical need to screen women for cervical pre-cancer lesion (Rositch *et al.*, 2011). The following sub-sections provide an

overview of each of these screening methods although screening by cytology and HPV testing were the only used screening methods in the study reported in this thesis.

2.9.2.1 Screening by Cytology

The commonest cytological method for cervical screening (detecting precursor lesions), referred to as conventional cytology, involves performing a Pap smear test. Briefly, this involves the collection of exfoliated cells from entire transformation zone of the cervix and the cervical canal (figure 2.1) using a spatula and/or cervical brush (Moss *et al.*, 2004). The collected cells (cervical swab sample) are then “smeared” uniformly on to a microscope slide, followed immediately by the application of a fixative. The smear is subsequently stained with dyes (Papanicolaou stain) and examined for indications of cellular abnormalities under a microscope (WHO; Chronic Diseases and Health Promotion Group, 2006).

The implementation of Pap smear, as an intervention/screening tool in well-organised national cervical cancer screening programmes, has had the most profound reduction in the incidence, prevalence and deaths related to cervical cancer; however, these have only been achieved in developed countries (Lăără *et al.*, 1987). For instance, between the years 1965 and 1982, a reduction in cervical cancer deaths of between 34% and 81% were observed among the Nordic countries that achieved nationwide coverage in their implementation of Pap smear based cervical cancer screening programmes (Lăără *et al.*, 1987). Additionally, the 1960 to 1980 screening programme carried out in Canada resulted in the reduction of cervical cancer incidence from about 28.4 to 6.9 per 100,000 women per year and a reduction in related mortality, from 11.4 to 3.3 per 100,000 women per year (Denny, 2012). The effectiveness of conventional cytology at this reduction has been due to its ability to detect

pre-cancer lesions (CIN2 and CIN3) with a sensitivity that ranges from 47% to 62% and a specificity that ranges between 60% and 95%, globally (Sankaranarayanan *et al.*, 2005).

Another cytology-based screening tool known as liquid based cytology (LBC) was developed as an improvement of the conventional cytology method (Moss *et al.*, 2004). Its sensitivity averages as 57% and specificity of about 97%, LBC has shown relatively small improvements on the sensitivity and specificity of conventional cytology. Although there were some controversies regarding the initial studies of the LBC technology, it is being widely used in the USA and UK for routine screening for cervical lesions (Moss *et al.*, 2004). However, like conventional cytology, LBC is currently difficult to implement in developing countries for screening programmes because it has similar requirements for implementation and so does not address the difficulties developing countries have with cytology based methods, such as, limited quality controls, limited instrumentations, high cost, limited trained personnel and inefficiencies in follow-ups (Denny, 2012). However, in studies (both in developed and developing countries), such as the one reported in this thesis, cytology testing is often used as gold standard for assessing other screening methods.

2.9.2.2 Screening by HPV Testing

The most recent addition to the options for cervical cancer screening has been the detection of HPV infection in exfoliated cervical cells collected for Pap smear testing. Following extensive evaluation in numerous studies, HPV test as a screening tool is acceptable in both developing and developed countries. These studies have consistently shown that HPV testing is more sensitive (between 84% and 90%) but less specific (between 70% and 86%) for the detection of pre-cancer lesion (CIN2 and CIN3 grade lesions) as compared to cytology based testing, which reported sensitivity of between 57% and 61% and specificity of between 82%

and 90% in the same group of studies (Patanwala *et al.*, 2013; Castle *et al.*, 2012; Cuzick *et al.*, 2006; Kulasingam *et al.*, 2002; Solomon *et al.*, 2001). Other important findings of these HPV studies were that HPV testing was more reproducible.

Subsequent studies with improved study designs and testing technologies (more sensitive molecular techniques) have also compared HPV testing to cytology based testing. These provided stronger evidence for the higher sensitivity but slightly lower specificity of HPV testing (Ronco *et al.*, 2010; Leinonen *et al.*, 2009; Mayrand *et al.*, 2007b; Ronco *et al.*, 2006). One of the largest comparative studies of cervical screening methods in a developing country was a randomised control trial conducted in India. In that study, it was shown that a single HPV testing was associated with a significant reduction in cervical cancer cases and related deaths relative to a control group; while cytology based testing on the other hand did not significantly reduce cervical cancer incidence or related deaths (Patanwala *et al.*, 2013; Castle *et al.*, 2012; Sankaranarayanan *et al.*, 2009).

In order to overcome the observed low specificity of HPV testing for the detection of CIN2 or worse lesions, concurrently HPV and cytology based testing for cervical cancer screening, known as co-testing, was evaluated (Dillner *et al.*, 2008). The findings of those studies indicated that co-testing of women by HPV and Pap testing was a useful approach for cervical cancer screening (Dillner *et al.*, 2008). One of the major studies that evaluated co-testing for cervical cancer screening, the so-called Joint European Cohort Study, reported that being negative for both HPV and Pap testing predicted the lowest risk for the development of cervical cancer (Dillner *et al.*, 2008). It was also observed that being HPV negative but Pap positive marginally increased the risk of the incidence of cervical cancer, which was at a constant rate over a period of six years. However, being HPV positive but Pap negative progressively increased the risk of cervical cancer incidence over the same period. Also,

being positive for both HPV and Pap testing was observed as the most significant predictor of cervical cancer incidence (Dillner *et al.*, 2008).

In routine real-world clinical practice, co-testing had similar predictive values as compared to the experimental study conditions. In one of the major studies that showed this (Katki *et al.*, 2011), the cumulative incidence of CIN3 or worse lesion over a five year period was 0.17% and 0.36% for women who tested negative by only HPV or Pap testing (respectively). For the women who tested positive by only HPV or Pap testing, their cumulative incidences were 7.6% and 4.7% respectively (Katki *et al.*, 2011). However, having a negative result for both HPV and Pap testing had a 0.16% cumulative incidence over the same period, indicating that performing both tests was more reassuring for women who had negative results, particularly compared to those with negative Pap results. The cumulative incidence for a woman with a negative HPV test but a positive Pap test only went up marginally to 0.86%, while having a positive HPV result and a negative Pap results increased the cumulative incidence to 5.9%. Furthermore, having positive results for both tests increased the cumulative incidence to 12.1% (Katki *et al.*, 2011). This clearly shows that having an HPV result makes a big difference for women who are Pap positive, that is, a risk of 0.86% if HPV negative or a risk of 12.1% if HPV positive. Additionally, some studies have shown that genotyping the detected HPV in order to identify high risk HPV (HR-HPV) type infections, particularly those of HPV-16, 18, 31 and 33 improves the positive predictive value of HPV testing for the detection of CIN 3 or worse lesions (Söderlund-Strand *et al.*, 2011; Castle *et al.*, 2005; Khan *et al.*, 2005). However, it is worth noting that current algorithms that include genotyping triages are targeted at women 30 years or older, because younger women often have transient HPV infection.

A number of co-testing implementation strategies have been evaluated globally, which has been summarised thus: an initial HPV testing followed by cytology triage of HPV positive women or an initial cytology testing followed by an HPV test triage of women with CIN2 or worse grade lesions (Patanwala *et al.*, 2013). The ATHENA HPV study, a randomized controlled trial, which evaluated nine strategies, including a triage with HPV genotyping, concluded that a screening strategy that will have an HPV testing triaged by a combination of HPV genotyping and cytology does well on the three major screening outcome measures; sensitivity, specificity, and reduction of potential harm (Cox *et al.*, 2013; Patanwala *et al.*, 2013; Castle *et al.*, 2012; Cuzick *et al.*, 2012; Castle *et al.*, 2011a). The FOCAL trial, another randomized controlled trial of HPV testing for cervical cancer screening using CIN3 or worse lesion as the end point, when completed and finally reported, is expected to provide data regarding the efficacy of two co-testing strategies; an initial hr-HPV DNA testing (genotyping) followed by liquid based cytology (LBC) triage of hr-HPV-positive women and an initial liquid based cytology (LBC) testing followed by hr-HPV triage (Ogilvie *et al.*, 2010).

Although testing technologies for HPV detection has rapidly improved, from the conventional PCR through the signal amplification technologies (as Hybrid Capture I and II) to the combination of multiplex, nested and real time PCRs, only the sensitivity of HPV testing has been improved, without an improvement of its relatively lower specificity. This is due to the fact that while the HPV test detects current HPV infections, which also includes those that are transient; it is rather persistent HPV infections that account for the incidence of cervical cancer. Therefore, newer molecular technologies for the detection of other viral biomarkers such as HPV RNA, E6 and E7 HPV proteins and related expressions, and non-viral biomarkers (such as p16INK4a and Ki67 antigens, methylation of *CADMI* and *MAL*

genes⁶) are rapidly being improved in order to be able to differentiate between persistent and transient HPV infections, with the possibility of an improvement of the lower specificity of HPV testing (Patanwala *et al.*, 2013; Cuzick *et al.*, 2012). The introduction of HPV testing in developing countries may face some of the problems associated with a national cytology-based screening programme such as the high cost of setting up infrastructure for call and recall, which remains very poor, and inefficiencies in follow-up.

The approach adopted in the study reported in this thesis was that the women who consented to participate were concurrently (co-tested) tested by Pap smear and samples from the spatula and brush were recovered for HPV DNA testing and genotyping.

2.9.2.3 Screening by Visual Inspection of the Cervix

A third major screening method for cervical cancer prevention involves the direct inspection of the TZ, SCJ and the external os with the unaided eye and a bright light after the application of either Acetic acid (VIA) or Lugol's Iodine (VILI). For VIA, the inspection is done at least 1 minute after a 3%-5% solution of acetic acid has been applied to the cervix and the appearance of a distinct acetowhite area in the TZ or near the SJC is looked for. In the case of VILI, the inspection is performed following the application of Lugol's Iodine and the appearance of a distinct yellow area in the TZ close to the SJC is indicative of a positive test.

These are very simple, affordable and easy to implement approaches in screening programmes, particularly for developing countries. The nature of these testing methods makes them amenable to what has come to be known as the 'single visit' or 'screen-and-treat' approach in cervical cancer prevention. With that approach, women with a positive test, without further triage, are directly treated by cryotherapy. The evaluations of these testing methods in many developing countries have shown that they reduce loss to follow-up, delays

in treatment and missed cases (Cuzick *et al.*, 2012; Sankaranarayanan *et al.*, 2005). Of the two testing methods, VIA has been the most evaluated, mostly in developing countries including Ghana, Thailand, India, South Africa and Zimbabwe. The sensitivity and specificity of these testing methods have varied greatly in most of the cross-sectional studies that evaluated them; they have both ranged between 14% and 95% with an average sensitivity of 50% and average specificity of 80% for the detection of CIN2 or CIN3 lesions (Cuzick *et al.*, 2012; Sanghvi *et al.*, 2008; Gaffikin *et al.*, 2007).

The major limitations of these testing methods are that the appearance of either the acetowhite or yellow area (for VIA and VILI respectively) may not be due to cervical neoplasia, since inflammation, regeneration of the cervical epithelium and immature squamous metaplasia give the observed colour changes, resulting in treatment of women who do not need any treatment (over-treatment). The results of these tests may be difficult to determine for postmenopausal women. Additionally, these test are predominantly unable to detect glandular precursor lesions, since it is difficult to visualize the endocervical canal. Issues, such as, the lack of a uniform criteria for reporting results, subjectivity in the interpretation of results, the need for frequent re-training, all result in the widely varying sensitivity and specificity of these tests (Cuzick *et al.*, 2012). Balancing the positives and limitations of this screening method, the VIA/VILI although may be easier to implement in resource limited countries, it is most likely going to lead to some appreciable level of over treatment and over referrals for colposcopy with accompanying increase in cost.

2.9.2.4 Self-Sampling and Participation in Screening

Although not a screening method, self-sample collection is a strategy developed such that women are able to collect their own samples which may be useful for both cytology and HPV

DNA testing. This strategy was developed to support the most sensitive and most specific screening methods (HPV DNA and Pap testing respectively), in order to overcome the burden of logistics and training needs associated with the implementation in developing countries of Pap and HPV testing. Additionally, self-sample collection strategy was developed to address the challenges that limit women's participation in screening activities, these include lack of time, discomfort, inconvenience, cultural objections, unwillingness and inability to travel to health facilities for Pap and HPV tests (Arrossi *et al.*, 2015; Virtanen *et al.*, 2011; Anhang *et al.*, 2005). Although most developing countries are evaluating and introducing screening based on VIA and VILI, as discussed earlier, these are not as sensitive and specific as Pap and DNA testing (Cuzick *et al.*, 2012; Petignat and Vassilakos, 2012) and therefore there is the need to study the usefulness and acceptability of self-sample collection.

In order that most women get tested and are able to benefit from the advantage of HPV testing, self-collected cervicovaginal cell samples for HPV testing have been evaluated relative to both HPV testing of health-personnel collected cervical samples and Pap testing. Findings of cross-sectional studies indicated that there was a high rate of agreement between health-personnel collection (HPC) and self-collection (SC) with regards to the HPV positivity. In one such study, an overall agreement of 88.1% with a kappa of 0.73 was reported; while HPV testing of SC did not detect HPV infections in 14 women who were HPV positive by the corresponding HPC, HPV testing of HPC samples also missed the HPV infection in 18 women detected as positive with SC. The statistically none significant discordance and other differences observed between the two types of samples was due to the fact that more low risk types of HPV were detected in SC than HPC specimen (Gravitt *et al.*, 2001). In more recent studies, overall agreements of between 91.8 and 93.6% were reported between the two sample types with kappa of between 0.67 and 0.73. The levels of

discordance were also similar. In one such study, HPV types were detected in 15 women using their HPC samples that were not detected by their SC samples. On the other hand, those detected in 17 women using their SC specimen were not detected in their HPC specimen. The rates of type-specific concordance in women with multiple infections were also fairly high (Petignat and Vassilakos, 2012; Zhao *et al.*, 2012; Bhatla *et al.*, 2009).

In respect of the sensitivity and specificity for the detection of CIN2 or worse lesions, generally SC was 10%-19% less sensitivity but of similar specificity relative to HPC. A study had reported even very similar sensitivity and specificity for both sample types; 93.6% and 82.5%, respectively for SC and 93.2%, and 87.5%, respectively for HPC. In the same study, the sensitivity and specificity for cytology were 77.5%, and 87.3%, respectively, indicating how similar SC is to HPC relative to cytology (Bhatla *et al.*, 2009). In a community based randomized controlled trial in Mexico, the so called MARCH trial, a relative sensitivity for the detection of CIN2 or worse lesions and invasive cancer were 3.4 and 4.2 times more respectively, for SC as compared to cytology although it recorded a lower positive predictive value and a lower specificity because it detected more low risk HPV types (Lazcano-Ponce *et al.*, 2011). In all, these reports show that self-collection is a viable alternative to HPC and complementary to cytology testing (Scarinci *et al.*, 2013; Belinson *et al.*, 2003). The fact that self-sampling does not need pelvic (speculum) examination, attendance at clinics or the presence of health personnel, and that it can be performed at the convince of a woman at home and the collected specimen may sent through the regular mail, makes it potentially a useful tool for increasing the coverage and participation of women in cervical cancer screening. It has been shown that the response to self-collection can be varying for different populations. For example, women who were non-attendees of regular cervical screening in Italy, Finland, Sweden and Netherlands responded to self-collection at the following rates,

19.6%, 31%, 39% and 34% respectively, which was more than the use of letter to remind non-attendees (about 10.0%). However, for all these studies, an average of 5% increase in overall screening coverage due to the introduction of self-collection were reported (Virtanen *et al.*, 2011; Rossi *et al.*, 2011; Gok *et al.*, 2010; Sanner *et al.*, 2009).

The overall influence of self-collection on cervical cancer screening has led to its recommendation as an alternative method of sampling in national screening programmes and most women in most studies including those referred to above, were of the view that self-sampling was easy to perform and that they did not experience difficulties with it. The study reported in this thesis applied two separate approaches without the provision of incentives; a hospital based approach and a community centred approach for self-collection. Detailed description of these approaches are provided in the method chapter of this report. The second approach was designed with the aim of overcoming the limitations of not meeting women at home and the difficulties women have when they needed to travel to the hospital for kits and for the return of their samples.

2.9.2. Summary of Prevention of Cervical Cancer

Primary prevention of cervical cancer, which was mainly by education on abstinence form causal sex and the promotion of the use of condom, was on a low key, however, the development of three vaccines against nine different genotypes has brought the primary prevention of cervical cancer to the fore. Research continues towards the development of new vaccines ('second-generation' vaccines) against the HPV types that are not protected against by the current available vaccines (Chatterjee, 2014). In spite of these improvements in primary prevention, secondary prevention which has mostly been by screening for cervical lesions (cytology tests) and lately screening for HPV infection (HPV DNA test) has been the major tool for reducing cervical cancer incidence and mortality. In the era of vaccination,

screening remains relevant as it serves as a means of assessing the success of vaccination programmes in preventing the development of cervical cancer precursor lesions among other important issues..



CHAPTER THREE

METHODS

3.1 STUDY LOCATIONS

On the basis of the following facts, that HPV is the commonest sexually transmitted infection (STI), that other STIs share similar sexual behaviour risk factor profiles (Burchell *et al.*, 2006) and that *Chlamydia trachomatis* (CT), herpes simplex virus type 2 (HSV-2) and HIV encourage the development of cervical lesions (Muñoz *et al.*, 2006; Samoff, 2005; Smith *et al.*, 2002a, 2002b, 2004) and HPV infections and persistence (Burchell *et al.*, 2006; Samoff, 2005), STIs may serve as good surrogate indicators of the risk of HPV infections in a community. Therefore, a high risk community in this study was defined as any district that recorded one of the highest STI prevalences in Ghana in the year 2010. The Lower Manya Krobo District of the Eastern Region was that district with the highest STI prevalence and therefore was selected for this study. Subsequently, the Akuse sub-district of the Lower Manya Krobo District (Figure 3.1) was randomly selected, among the 5 sub-districts by random number selection using the Ms. Excel software, for the conduct of this study.

The Akuse sub-district is made-up of 18 communities, 12 of which are located closely (centred at the coordinates 6°06'02.43N and 0°07'16.87E), while the other 6 are between 3 km and 5 km west of the 12 communities. The sub-district has a Government District Hospital with some of the communities having a Community based Health Planning and Services (CHPS) Centre. As at the time of the design of this study (December 2010), the available population data for the Akuse sub-district was the Ghana 2000 census's estimate of population size for the year 2011 (the 2011 estimates were found not to be substantially

different from the 2010 census data when it became available). The population of the Akuse sub-district was estimated to be 8887 (for 2011), of which 29.2% (2595 women) were females between the ages of 15 and 65 years (Ghana Statistical Service, 2000). There were four common Ghanaian languages spoken within the 18 communities of the sub-district; these were Hausa, Ewe, Ga-Adangbe and Twi. The study questionnaire was pre-tested in the Atua community of the Odumase sub-district.

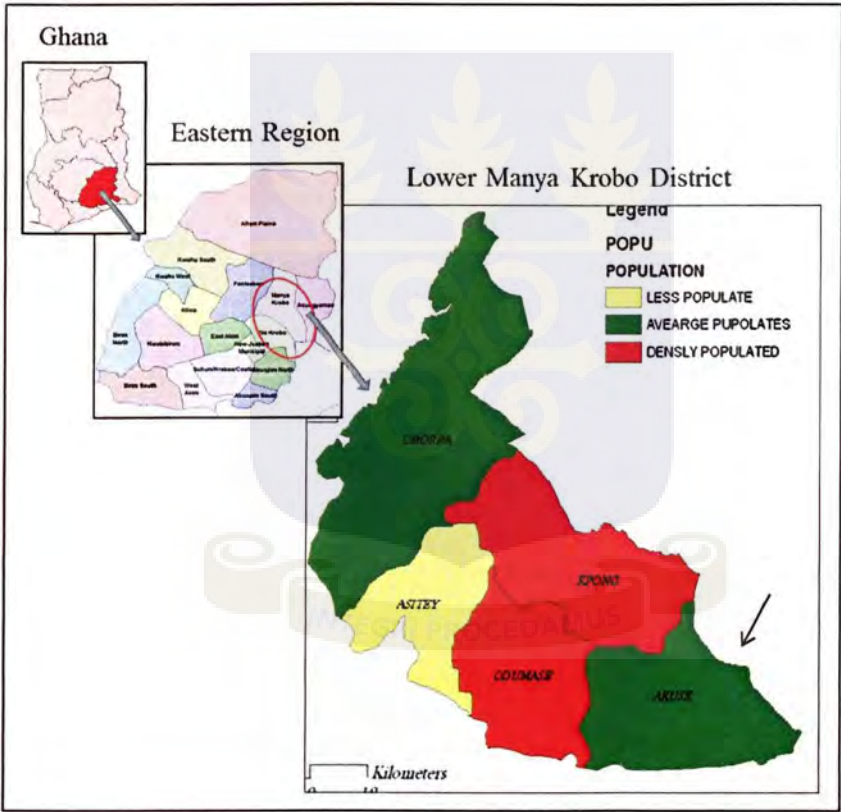


Figure 3. 1 Population distribution in the Lower Manya Krobo District.

The sub-district selected for the study is indicated by the small arrow.

3.2 SOURCE AND STUDY POPULATION

Women living within the Lower Manya Krobo District between March 2012 and March 2013 were targeted in this study, however, those who were living in the communities of the Akuse sub-district within this period were recruited to participate in this study.

3.3 SAMPLE SIZE DETERMINATION

A minimum sample size was calculated based on the random sample size equations and a modification (S) since the population was less than 10000.

$$N = \frac{Z^2 p(1-p)}{d^2}, \quad S = \frac{N}{1 + \frac{N}{Population}}$$

N= sample size.

Z= the standard normal deviation (set at 1.96 corresponding to the 95% Confidence Interval).

p = the proportion in the target population estimated to have an HPV infection (HPV prevalence).

d = degree of accuracy desired or the maximum difference to detect

(Expected prevalence – Worst acceptable prevalence).

Since there were no data for the prevalence of HPV infection in the general population of any community in Ghana, the HPV prevalence (p) reported in a Nigerian community (26.3%) and

neighboring West African countries with the highest and lowest reported HPV prevalences were used. The degree of accuracy (d) was set as 0.065, which is the closest to the average difference between the HPV prevalence in West African countries (Jemal *et al.*, 2012; Piras *et al.*, 2011; Vaccarella *et al.*, 2008; Thomas *et al.*, 2004). Since the study was a cross-sectional study, a design factor of 1.2 was applied to obtain a sample size of 211 participants. A response rate of 60% was expected, based on literature with respect to response to cervical screening and the sample size was adjusted by this rate to obtain the target of 352 women for the study.

3.4 STUDY DESIGN (CROSS-SECTIONAL STUDY)

3.4.1 Study Type, Selection of Communities and Distribution of Sample Size

A cross-sectional study design was used for this study. Of the 5 sub-districts of the Lower Manya Krobo District (figure 3.1), one (the Akuse sub-district) was randomly selected by the use of the random number function in Ms. Excel software. Specifically, the names of the five sub-districts were listed, not in any order, and numbered 1 to 5. The Excel formula “=RANDBETWEEN(1,5)” was used to randomly select one of the numbers from 1 to 5, and that which corresponded to the number assigned the Akuse sub-district on the list (3) was returned. All its communities included in the study. Based on the population of women between the ages of 15 years and 64 years in each community, the number of women targeted for the study (352 participants) was distributed among the communities by the probability proportional to size (PPS) method. That is, the population of women (older than 15 years) for each of the community was determined and the sum calculated. The ratio of the population for each community to the sum of the populations was determined and the resulting fraction

was used to multiple the number of women targeted for the study in order to obtain the number of women targeted for each community.

3.4.2 Community Entry and Engagement

For this study location, it was found that the Akuse Government Hospital was the most influential institution through which the community could be successfully engaged. Therefore, with the assistance of the hospital administration, a meeting involving the religious leaders of the orthodox churches, researchers and the hospital administration took place. At this meeting, a community education programme was planned. Presentations on cervical cancer and the study were made in two churches during the Christian Home Week Celebration, (during this celebration, members of all the orthodox churches in the sub-district meet in one of the member churches for specific activities). Also, announcements were made regularly at all the orthodox churches regarding the study and its presence in the communities. A second presentation was made mid-way through the field work in a different church during a women's group celebration. The leaders of the Muslim community were separately contacted and during a Friday Muslim prayer service the community members were informed of the study and issues relating to cervical cancer were discussed.

Each of the communities in the sub-district was entered by contacting the traditional leaders of the respective community with the assistance of the Public Health Nurses. The community members were reached by the traditional system of announcement and the activities of the Community Health Volunteers within those communities. The participants were subsequently recruited by house visits. The Public Health Nurses and the Community Health Volunteers engaged the communities and participants continuously during their regular community outreach programmes. They explained the study and provided information about cervical cancer as well as taking feedbacks from the study team to the members of the community and

the participants. This study completely avoided the involvement of the political leaders and/or members of the local government system because of the existence of some political disputes and legal issues within the sub-district to ensure freedom and willingness of participation.

3.4.3 Sampling and Reporting Strategies

The study was designed to employ a single reporting strategy for the participants, described below as reporting strategy 1 (section 3.4.3.1) and depicted as figure 3.2a. However, due to an initial low reporting rates, which resulted from women not able to make time off their daily and economic activities to go to the hospital during working hours for specimen collected, a discussions with mostly the women who were not reporting (by phone calls), the Nurses of the Public Health Unit and the Community Health Workers of the Akuse Hospital, resulted in the gathering of important information and suggestions. Based on these information and suggestions, a new reporting strategy was developed that improved the reporting rate. This is described below as reporting strategy 2 and depicted in figure 3.2b. Another recruitment/reporting strategy that evolved during this study was what was referred to as peer-recruitment (described below as additional observation). Women recruited by this means were allowed to participate, so that the study may be able to understand how what women within a community say among themselves regarding a screening programme, may influence the overall participation of women in cervical cancer screening.

3.4.3.1 Reporting strategy 1

Women were identified by a house survey of 17 of the 18 communities in the Akuse sub-district. In each of those communities, the major roads/paths through it were identified by the use of Google Map computer application and grouped in order of direction, from one end of

the community to the other. Based on the crude estimate of the average number of houses and the population of women in each of the communities, it was assessed that visiting every 5th house or less will ensure a wide coverage of the communities, therefore a number was selected from among the numbers 1 to 5, which was 3 to represent the random count of houses to visit. Therefore, starting from the first selected road/path, every third house to the right and left of the road/path was visited. The women in each house meeting the inclusion criteria were invited to participate in the study after explaining the study, the consent form and the options available to them as participants as well as giving them basic information about cervical cancer. Each consenting woman was assisted by a researcher to complete the study questionnaire. The women were given the option to set a date (a period within a week or more than one week) they were going to come to the hospital for a dual specimen collection, which were self-specimen collection performed by the women and Trained Health Personnel collected specimen. This was repeated for each road until the desired sample size was attained for each community. Women, who had not reported to the hospital for more than two weeks after the date they opted for, were called on the mobile phone number(s) they gave, until they were reached once, they were reminded and a new date arranged for them to visit the hospital for sample collection. These are illustrated in figure 3.2 below.

3.4.3.1.1 Peer-recruitment

It was observed that some women who reported for sample collection at the hospital were not recruited by the researchers, but were encouraged by the women who lived with them (in the same house), who met the researchers during the house survey. For each of the houses surveyed that had less than three women recruited by the researchers, the peer invited /women were allowed into the study and treated as was done for the other women recruited by the researchers. That is, the study was explained to them, those who were willing

provided informed consent and the study questionnaire was administered as described above. Specimens were subsequently collected in the same way as described for the other women.

3.4.3.2 Reporting Strategy 2

This field-developed approach (figure 3.2b) involved identifying, a location within a community where a room was available for the women to be attended to, in privacy. This location was mostly central, well known and convenient to quickly reach from all other parts of the community. Women recruited by the house survey were invited to participate by going to the selected location at any time between 9:00 am and 7:00 pm. Women who reported and met the inclusion criteria were invited to participate in the study after administering the consent process as described above. Consenting women were assisted by the researcher to complete the study questionnaire.

Since this approach evolved on the field, only 4 of the 17 communities that had not yet been involved in the study at all, were reached with this approach and as such the study was not sufficiently powered to determine the differences in the reporting rates between this and the original approach. By the nature of this second approach (particularly the fact that there was more time to report within a day and that the completion of questionnaire was at the collection point but not at home during recruitment), the level of motivation for the women in these 4 communities were different from those who were to report at the hospital. Therefore, differences in performance between this and the other approach are mainly suggestive of their relative potential influence of cervical cancer screening activities in Ghana and other developing countries with similar settings.

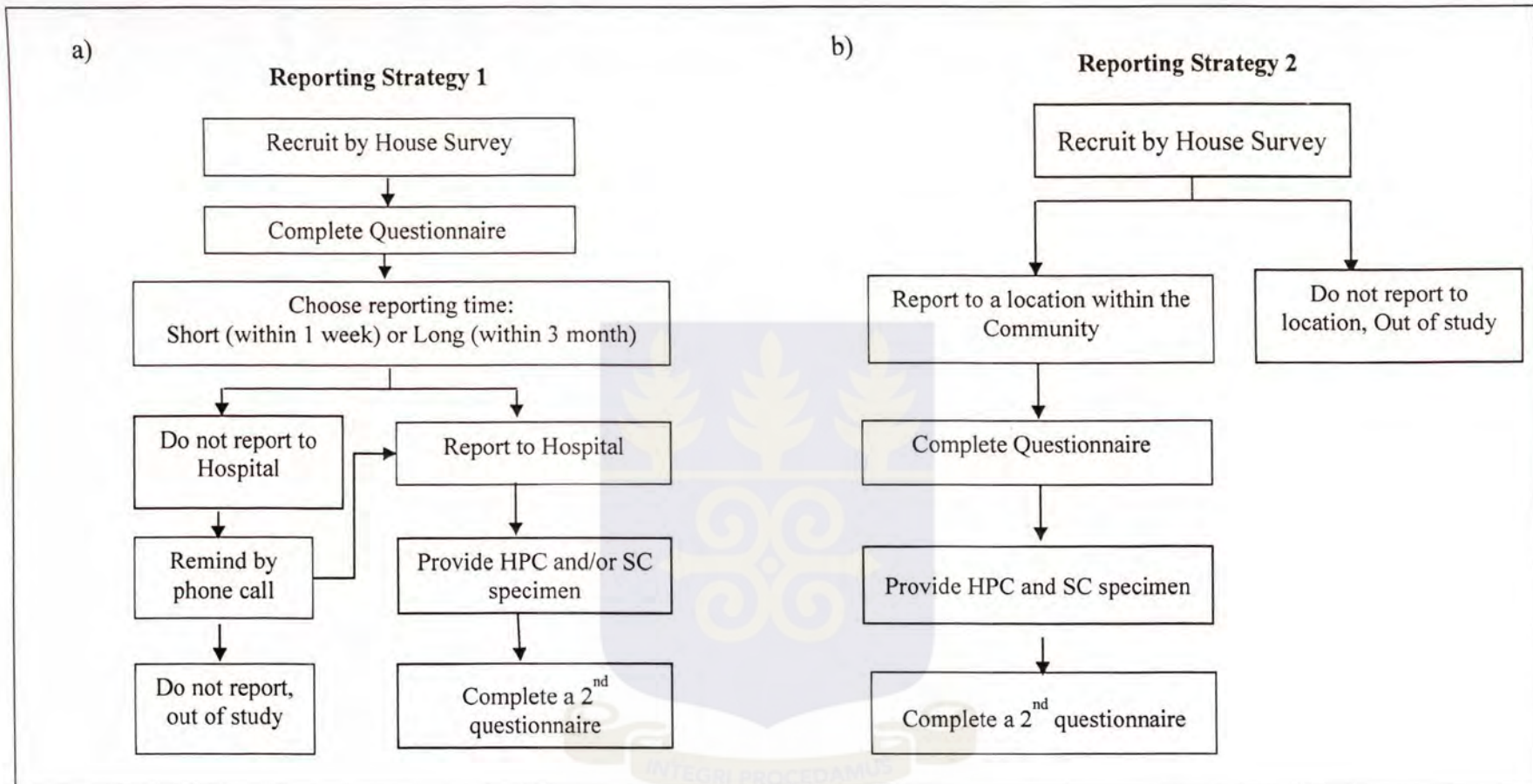


Figure 3. 2: An illustration of the design for reporting for specimen collection.

3.4.4 Inclusion and Exclusion Criteria

Self-reported healthy women between the ages of 15 and 70 years who were willing to provide specimen by at least one of the two specimen collection methods were included in this study. Women who had undergone hysterectomy or cervical conisation were excluded from the study. Women who reported they had never had sexual intercourse were also excluded from the study. Self-reported pregnant women were also excluded from the study; this was because of the controversial nature of cervical screening during pregnancy and the occurrence of maternal anxiety and the potential of association of participation in screening with any future occurrence related to the pregnancy (Flannelly, 2010). Additionally, there is a high potential of over- and underestimation of cytological changes or possible lesion which may have been due to the normal physiological changes associated with pregnancy (Flannelly, 2010; Hunter *et al.*, 2008; Van Calsteren *et al.*, 2005).

3.5 ETHICAL ISSUES

The study was approved by the Ethics Review Board of the Ghana Health Service (ID No GHS-ERC: 06/11/10). For the participants who were able to read and understand English, they read and signed an informed consent form (in English) that explained the main research goals, sample collection procedures, potential benefits and harms, and privacy and confidentiality of data collected for the study. For those who could not read English, the informed consent form was

translated to a local language and explained to them; each of the four languages spoken in the communities (Hausa, Ewe, Ga-Adangbe and Twi) was translated by one particular researcher throughout the study. Each woman was given a card indicating her community, date of recruitment, a short title of the study and the place to report for sample collection. Questionnaires were administered by a one-on-one interview, in such a manner as to ensure that no other person heard the participant and the researcher. The description of the process for self-collection of cervical swabs (self-specimen collection) was also done one-on-one and with the aid of pictures/illustrations. The self-specimen collection was done by the participants alone in a separate room and with the illustrations of how to perform the process in hand. Subsequently, a vaginal examination and health-personnel sample collection was performed with only the health personnel (Medical officer or Nurse) and the participant behind a screen in a room privately. Since no woman was found to have intraepithelial lesions or Pap positive results, there were no referrals for subsequent management. However, women HPV positive were advised to re-test after 3 years.

3.6 QUESTIONNAIRE ADMINISTRATION

The study questionnaire was finalized after the pre-test, in the Atua Community, Odumase sub-district. Two trained Public Health Nurses assisted the study participants, by a one-on-one interview, to complete a study questionnaire (appendix I). This was used to collect information on the socio-demographic characteristics, sexual behaviour, sexual and reproductive history, menstrual factors, use of oral contraception, and history of sexually transmitted infections and cervical cancer screening history.

3.7 SAMPLE COLLECTION AND SMEAR PREPARATION

Each participant was given the option to choose to perform both SC and HPC or any one of the two as they wished. However, for the most of them who choose to perform both, they performed the self-collection followed by the Health-personnel collection. This order, as has been reported by most studies which made both methods available, was due to the advantage of identifying women who may have been within the period of menses and should be excluded from the Pap test (Lazcano-Ponce *et al.*, 2011; Adler *et al.*, 2012; Bosgraaf *et al.*, 2014; Gravitt *et al.*, 2011; Bhatla *et al.*, 2009; Bais *et al.*, 2007)). To collect the sample(s), each participant, one at a time, was instructed on how to use the Rover® Viba-Brush vaginal sampler, (Rovers Medical Devices, The Netherlands), using the illustrations on the packaging of the brush. The women were directed to a private room and reminded to wash their hands before using the kit. The collected specimen, on the detachable part of the brush, was transferred into a sterile 15 mL screw-capped tube containing 5 mL PBS with 5 mM ethylene diamine tetraacetic acid (EDTA), pH 8.0. These specimens were designated as self-collected (SC) for HPV testing. Women having their menses were asked to come back one week after the end of their menstrual period.

The nurse practitioner then performed a standardized pelvic examination. With the aid of a sterile plastic disposable speculum, the same trained Nurse, used the PAP-PAK® Cytology kit (Medical Packaging Corporation, Camarillo, USA) to collect cervical specimen, according to the manufacturer's instructions, for Papanicolaou (Pap) smear and HPV testing. Briefly, the head of a wooden Ayre's spatula was used to collect cellular material from the cervix and the squamocolumnar junction, and then a cytobrush was used to collect cellular material from the endocervix. These were smeared uniformly on separate sections of a microscopic slide (for Pap

testing) followed by the application of a fixative (95% ethanol and 5% polyethylene glycol) provided with the kit. The cytobrush was then rinsed in 50 μ L of DNAgard[®]Tissue (Biomatrix, Inc., San Diego) in a 2 mL screw-capped micro-centrifuge tube. Also, the head of the wooden spatula was broken and added to the same 2 mL screw-capped tube to complete the sample used for HPV testing. These specimens were designated as Health Personnel Samples (HPS). All specimens for HPV testing were transported at 4 °C and store at -20 °C until used for HPV testing.

3.8 PAPANICOLAOU STAINING OF SMEARS

The slides were completely immersed in two changes of 95% ethanol to remove the carbowax in the fixative, overnight. The slides were thereafter placed in racks with a continuous flow of running water to hydrate for 5 minutes and then stained with Mayer's Hematoxylin for 5 minutes. The slides were washed in running water for 5 minutes, after which they were dehydrated by immersion in 95% ethanol for 2 minutes. The slides were passed through Orange G-6 for 2 minutes, followed by two rinses of 95% ethanol, each time for 10 seconds. The slides were then transferred into a solution of EA for 3 minutes and rinsed very well with two changes of 95% ethanol, each time for 10 seconds. Dehydration was completed in absolute ethanol for 1 minute. The slides were air dried on the bench, cleared in xylene and coverslip fixed with DPX mountant (GCC Diagnostics, Gainland Chemical Co., Sandycroft Flints, UK). The slides were then examined under an Olympus BX51TF microscope with an Olympus DP20 camera (Olympus Corporation, Tokyo, Japan), for cellular characteristics.

3.9 DNA EXTRACTION

The volume of each specimen collected by HPS was made-up to 1 mL with PBS and vortex thoroughly. For each of both specimen (SS and HPS), 200 μ L of the cell suspension were used for DNA extraction on the MagNA Pure LC automated system (Roche Molecular Systems, Inc., Pleasanton, USA) using the MagNA Pure DNA Extraction Kit (Roche Molecular Systems, Inc., Pleasanton, USA) according to the manufacturer's specification with a modification of an addition of 15 μ L of RNase to the lysis buffer for each specimen. The negative controls included in each set of extraction were PBS, DNase/RNase free water, and DNA Gard, while the positive control was a suspension of SiHa cells with an integrated HPV 16 genome. The specimen/DNA quality was assessed by the amplification of the human RNase H gene in 10 μ L of DNA extract and analysed with Light Cycler 480 and related software (Roche Molecular Systems, Inc., Pleasanton, USA).

3.10 HPV GENOTYPING

3.10.1 Nested-Multiplex PCR

For each specimen, 15 μ L of its extracted DNA were used in amplifying the HPV L1 gene with the consensus PGMY09/PGMY11 primer sets (sequences presented in Table A in the Appendix) in a first round PCR. In addition to the 15 μ L of extracted DNA, the 50 μ L final reaction volume contained 200 μ M of each of the four dNTPs, 1X PCR Buffer (200 mM Tris-HCl pH 8.4; 500 mM KCl), 6 mM of $MgCl_2$, 0.6 μ M of each of the 18 PGMY primers and 1.25U of Taq DNA polymerase. The PCR conditions, for the first round of nested PCR were, an initial denaturation step at 95 °C for 9 min, followed by 40 cycles of, denaturation at 95 °C for 30 seconds,

annealing at 55 °C for 30 seconds and elongation at 72 °C for 30 seconds. This was followed by a single final extension at 72 °C for 5 min before holding the temperature at 4 °C.

The second round of the nested PCR was with 2 µL of the first round PCR products as DNA template and the GP5+/GP6+ primer set, (5'-TTTGTACTGTGGTAGATACTAC-3') and (3'-CTTATACTAAATGTCAAATAAAAAG-5') respectively. The GP6+ primer (Invitrogen, Burlington, Canada) carried a 5' biotin label and phosphorothioate bonds in the first 4 nucleotides on the 5' end. The 50 µL final reaction volume contained, 200 µM of each dNTP, 1X PCR Buffer (200 mM Tris-HCl pH 8.4; 500 mM KCl), 4 mM of MgCl₂, 1.0 µM of GP5+ primer, 1.0µM of GP6+ primer, 1.25U of Hotsat Taq DNA polymerase. The amplification was achieved with the following conditions, an initial denaturation step at 94 °C for 5 minutes was followed by 30 cycles of denaturation at 94°C for 30 seconds, primer annealing at 40 °C for 20 seconds and an elongation at 72 °C for 30 seconds. This was followed by a 7 minute final extension at 72°C and holding the temperature at 4°C.

3.10.2 Detection of PCR Products / Genotypes

The second round PCR products were used for the detection of 46 genotypes of HPV (HPV 16, 35, 40, 45, 58, 18, 66, 59, 6, 31, 74, 42, 52, 51, 62, 81, 83, 72, 90, 67, 53, 56, 33, 70, 87, 39, 30, 54, 44, 86, 82, 84, 68, 69, 85, 91, 73, 43, 11, 13, 32, 61, 71, 89, 97 and 26) by the map technology, as described by Zubach *et al.*, (2011). This involved an initial coupling of microspheres labelled (according to the manufacturer's instructions) with different ratios of red and infrared fluorophores (Luminex Corporation, Austin, TX) to HPV type-specific probes carrying a 5'-C12 amino modification that reacted with the carboxyl groups on the microspheres. The double stranded second round PCR products were linearized in 96-well PCR microplates



(Fisher, Ottawa, ON, Canada) with 2 μL of bacteriophage T7 gene6 exonuclease (New England BioLabs, Pickering, ON, Canada) that removed the non-labelled strand after 40 minutes incubation at room temperature. The PCR microplates were kept at 95 °C for more than 10 minutes while adding 33 μL of the microsphere mix (15 microspheres/ μL for each set) to each of the wells of each PCR microplate. These were incubated for hybridization at 60 °C for 10 minutes. After this, 25 μL of a 0.04- $\mu\text{g}/\mu\text{L}$ solution of streptavidin-phycoerythrin (PE) (Invitrogen) in 1-tetramethyl ammonium chloride (TMAC) (Sigma), were added and incubated for 5 minutes at 60 °C. Genotype specific hybridization was detected on a Luminex Liquid Chip 200 flow cytometer (Qiagen) using the Luminex IS software (Luminex). The analysis was carried out at 60 °C with a maximum volume of 50 μL of the sample and a minimum count of 100 microspheres per type, with settings of 8,300 and 16,500 for the lower and upper gates, respectively, as recommended by the manufacturer.

3.11 HPV 16, 18 AND 45 VARIANT ANALYSIS

The specimens positive for HPV16, 18 and 45 infections were selected for variant analyses since these are the known most oncogenic HPV genotypes and are the genotype whose variant analysis is important for understanding the contribution of variants on carcinogenesis of an HPV type. The long control region (LCR) of the HPV16, 18 and 45 genomes were separately amplified by a nested PCR and second round products sequenced.

3.11.1 HPV16 nested PCR

Using 5 μL of DNA extracted from each of the specimens, the first round PCRs were performed with the following primer sets; HPV16R1F (5'CACCTACTAATTGTGTTGTGG3'), HPV16R1R (5'GTTTGCACACCCCATG3'). The 50 μL final reaction volume also contained,

400 μ M of each dNTP, 1X PCR buffer (200 mM Tris-HCl pH 8.4; 500 mM KCl), 2.5 mM of MgCl_2 , 1.25 μ M of each of the two primers, and 5U of AmpliTaq DNA polymerase. The amplification was achieved with the following conditions; an initial denaturation step at 95 °C for 5 minutes was followed by 60 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 65°C for 30 seconds and an elongation at 72 °C for 30 seconds followed by a 7 minute final extension at 72 °C and holding the temperature at 4 °C. Extracted HPV16 DNA positive control and PCR water negative controls were used in each set of amplification.

The second round PCR used 5 μ L of the first round product and the following primer sets HPV16R2F (5'GGGGTACCTCGGTTGCATGCTTTTGGC3') and HPV16R2R (5'GGTCTAGACGGTTTGCACACACCCATGT3') for the amplification. The 100 μ L final reaction volume contained 200 μ M of each dNTP, 1 X PCR buffer (200 mM Tris-HCl pH 8.4; 500 mM KCl), 2.5 mM of MgCl_2 , 1.25 μ M of each of the two primers, and 6.3 U of Taq DNA polymerase. The amplifications were achieved with the same cycling conditions as that of the first round PCR.

3.11.2 HPV18 nested PCR

The nested PCR for HPV18 positive specimen used the same concentrations and cycling conditions as those for HPV16 except that the following primers sets HPV18R1F (5'GTGTTTGTGGTATGGGTGTT3') and HPV18R1R (5'GTATAGTATGTGCTGCCCAA3') were used for the first round and HPV18R2F (5'CGGTTGCATAAACTATGTAT3') and HPV18R2R (5'TCGGTTGCCTTTGGCTTATG3') were used for the second round. The controls used were a plasmid containing an HPV18 DNA positive control and PCR water negative controls.

3.11.3 HPV45 nested PCR

Using 5 µL of DNA extracted from a specimen each time, three separate first round PCRs were performed with the following sets of primers, for fragment 1 HPV45F (5'GACCTCGTAAGCGTCCTGC3') and HPV45R 5'GGATGCTGTGTAGTATG CAAGTTTATA3'); for Fragment 2 HPV45F2 (5'GTATGGTGTACTGTACATA ATTGTGG3') and HPV45R2 (5'CATAGGGTGTGGATACAGTTGTG3') and for Fragment 3 HPV45F3 5'CTGGCACATTTACAACCCCTAC3') HPV45R3 (5'CGCCAT CCTGCAATGCAC 3'). The 50 µL final reaction volume also contained, 400 µM of each dNTP, 1X PCR buffer (200 mM Tris-HCl pH 8.4; 500 mM KCl), 3.0 mM of MgCl₂, 1.25 µM of each of the forward and reverse primers, and 5U of AmpliTaq DNA polymerase. The amplification was achieved with the following conditions an initial denaturation step at 95 °C for 10 minutes was followed by 40 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 55 °C for 30 seconds and an elongation at 72 °C for 30 seconds. This was followed by a 7 minute final extension at 72 °C and holding the temperature at 4 °C. Extracted HPV45 DNA positive control and PCR water negative controls were used in each set of amplifications.

For the first round amplifications that did not yield enough PCR products for sequencing, a second round PCR was performed that used 2 µL of the first round product and the same primer sets as for the first round amplification. The 50 µL final reaction volume contained 400 µM of each dNTP, 1 X PCR buffer (200 mM Tris-HCl pH 8.4; 500 mM KCl), 2.5 mM of MgCl₂, 1.25 µM of each of the two primers, and 6.3 U of Taq DNA polymerase. The amplifications were achieved with the same cycling conditions as that of the first round PCR.

3.11.4 Visualization of second round PCR product

For all the second round PCR products of HPV16 18 and 45, 20 μL were resolved on a 1.5% agarose gel in 1X TBE buffer with 1 $\mu\text{g/mL}$ ethidium bromide at 120 V for an hour. The gels were documented with GelDoc™ XR+ System (Bio-Rad Laboratory, Mississauga, Canada).

3.11.5 Sequencing of second round PCR product

The second round PCR products were purified with the Amicon Ultra centrifuge filers (Millipore Ireland Ltd, Tullagreen, Ireland) with slight modification of the manufacturer's instruction. Specifically, a filter column was placed upright, into a micro-centrifuge tube for each sample, after which 200 μL of PCR water and 80 μL of a PCR product were added. The assembly was spun at 14000xg for 12 minutes. Additional 100 μL of PCR water were added to the filter columns. The filter columns were thereafter transferred, inverted, to a new set of micro-centrifuge tubes and spun for 2 minutes at 1000xg. The columns were discarded and the eluted DNA in each tube was quantified at 230 nm using NanoDrop ND-100 spectrophotometer (Thermo Fisher Scientific Inc, Wilmington, USA). The eluted DNA was diluted to 50 ng/ μL and sequenced with ABI Applied Biosystems 3730xl DNA Analyzer BigDye® Terminator v 3.0 (Applied Biosystem, Canada).

DNA Sequences obtained with the forward and reverse primers were assembled with SeqMan Pro software (DNASstar). The sequences obtained for HPV16, HPV18 and HPV45 PCR products were aligned to HPV16, HPV18 and HPV45 reference sequences (listed in Table S1) by the Cluster W method, using the MEGA 5.2 software. Phylogenetic trees were constructed based on the Test Neighbour Joining using the MEGA 5.2 software

3.12. STATISTICAL ANALYSIS

3.12.1 Data Entry and Quality Control

Double data entry was performed in SPSS version 20.0 (IBM Corporation, USA), after which data validation and cleaning were performed by merging the two data entries and checking for duplicates, illegal entries, non-entries, inconsistent coding and extreme/unreasonable values. SPSS version 20.0 and OpenEpi software were used for further data analyses.

3.12.2 Main Analyses

Initially, the data on the demographic characteristics, sexual and reproductive risk characteristics were described with frequencies, percentages and descriptive statistics. Additionally, the distributions of these were presented showing sub-categories for each.

Furthermore, in order to understand the influence of these characteristics (demographic, sexual and reproductive) on the reporting for specimen collection, a Chi-square analysis was used to determine the association between these participant characteristics and reporting for specimen collection, with special interest for the association with the inconsistent use of condom. It must be noted that the analyses of the relationship with reporting status excluded data on demographic characteristics and reporting status of the 38 peer-recruited women. This helped identify which category of women should be encouraged the more to participate in cervical screening. In other words, to determine which characteristics were associated with non-reporting. The relationship with condom use was assessed to inform the identification and targeting of education on condom use to the appropriate category of women; depending on which demographic characteristic and/or risk characteristics were more associated with inconsistent condom used. It must be noted

that the analysis excluded data on the women who did not report and the 38 peer-recruited women.

Following the observations of the distribution of the participants' risk characteristics, which are indicators of potential burden of HPV and cervical lesion, and in order to determine and confirm the burden of HPV, the overall, genotype specific, age-specific, high risk, low risk, multiple and single HPV infection prevalences for each specimen collection method, self-sampling (SC) and health personnel sampling (HPC), were described as proportions of the total number of participants tested for HPV within each category, and reported with a 95% confidence intervals.

Having used two methods for specimen collection for HPV burden determination, a comparison of the methods was necessary to inform future choice of method for screening. Therefore, the levels of agreement between the two specimen collection methods, in respect of each of the HPV infection prevalences, were analysed for using the Cohen's Kappa analysis and a determination of the proportions of concordance of HPV detection per woman, using only data of women who provided specimen by both SC and HPC methods was made. A McNemar's test was used test for the significance of the marginal proportion (concordance and discordance). Additionally, the age-specific prevalences for each of the specimen collection methods were obtained as a proportion of women, within each age-group that tested positive for HPV and these were compared and presented as paired line plots. Further to the comparison between the methods, an assessment of the strength of the association between sexual risk characteristics with HPV positivity for each specimen collection was performed by the use of logistic regression, to determine crude odds ratios (OR).

On the other hand, some analyses were not possible to perform, although intended, due to non-detection and limited power of the study. These include, all analyses related to the presence of cervical lesions (prevalence of and associations of cervical lesions with HPV positivity and known risk factors); these were not performed since no cervical lesion was detected in this study. Furthermore, this study was not powered to determine the magnitude of the association between the known sexual risk factors and HPV positivity (the sample size was smaller than needed for determining such associations), therefore this was not performed.

3.12.3 Additional Analyses

In addition to the analyses directly related to the specific objectives of this study, as indicated in section 3.12.2 above, analyses of additional collected data were performed and reported as additional findings.

- Chi-square analyses were performed to determine which demographic characteristics and known risk factors (excluding data of women who did not report for specimen collection and those recruited by peers), were associated with the stated post-performance preference for each of the two specimen collection methods used in this study. This will help identify what category of women who may prefer each of the specimen collection methods.

3.13 DEFINITION OF VARIABLES

3.13.1 Measures / Variables of Interest

The main outcome variables assessed in this study were as follows:

1. The detection of an HPV infection with each of the two specimen collection methods. This included the prevalence of HPV categorized as follows; overall, genotype specific, age-specific, high risk type, low risk type, single and multiple HPV infections,
2. The extent of agreement between the two specimen collection methods in respect of HPV positivity. Specifically, these included the Kappa values and the proportion of concordances of the overall, type specific, age-specific, high risk type, low risk type, single and multiple HPV infections.
3. The variant types of the detected HPV16, 18 and 45 genotypes.
4. The prevalence of cervical lesions (cytological abnormalities).
5. The distribution or proportions of the participants within the high risk and low sub-categories of each of the known sexual and reproductive risk factors. The high and low risk sub-categories of each of the risk factors are described under section 3.13.2 below.

Additional findings were also reported for this study, these were, the reporting rates for the different reporting strategies that evolved during this study, the levels of both post and pre-performance preferences of the two specimen collection methods and the associations of demographic characteristic and risk factors with condom use.

3.13.2 Independent Variables

For the analysis of the associations of sexual risk characteristics with condom use and with HPV positivity, the following were defined as the independent variables:

- Age at first sexual intercourse, as a risk factor for the acquisition of an HPV infection, was defined as the calendar age at which a woman first experienced penetrative vaginal

sexual intercourse. The AFSI sub-category, less than 20 years, is known to be high risk for HPV infection while the AFSI sub-category, greater 20 years, is known to be low risk for HPV infection (Erickson *et al.*, 2013; Bahmanyar *et al.*, 2012; Berrington de González *et al.*, 2004).

- Sexual age, as a risk factor for the acquisition of an HPV infection, was defined as the difference between the current calendar age of a woman and her calendar age at first sexual intercourse. The sexual age sub-category, <11 years is known to be high risk for HPV positivity while the category 11 to 19 years is known to be moderate risk and the category > 20 years is known to be protective of HPV infection (Lenselink *et al.*, 2008).
- Lifetime number of male sexual partners (LNSP), as a risk factor for the acquisition and persistence of HPV infection, was defined as the number of males a woman had had penetrative vaginal sexual intercourse with in her lifetime. The LNSP sub-category, ≥ 3 is known to be high risk for HPV positivity and progression of cervical lesion, while the sub-categories of less than 3 are known to be protective of HPV infection (Erickson *et al.*, 2013; International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Shields *et al.*, 2004).
- Consistent use of condoms during sexual intercourse, as a risk factor for the acquisition of an HPV infection, was defined as the use of condom by either the male or female partner during penetrative vaginal sexual intercourse. The inconsistent use or not of condoms are known to be high risk for HPV positivity, while the consistent use of condom is known to be protective of HPV infection.
- Number of pregnancies, as a risk factor for the acquisition of an HPV infection and the progression of cervical lesions, were defined to include all the pregnancies the woman

had carried, both full term or otherwise. The sub-category of > 4 pregnancies are known to be high risk for HPV positivity and progression of cervical lesions, while the sub-categories of < 5 pregnancies are known to be of low risk.

The general confounding factors including age, educational status and marital status were included in the analysis of the associations with condom use.

- Educational status, was defined as the highest attained educational level as at the time of the study and each sub-category included different levels of both the older and new educational systems in Ghana.
 - No-formal education referred to not having a formal education.
 - The Primary Category included the primary levels of both the older and new educational system.
 - Junior Secondary Category included the Middle school level and the Junior Secondary level of the older systems, as well as the Junior High level of the current educational system.
 - Senior secondary category included secondary level and senior secondary levels of the older systems, as well as the senior high levels of the current educational system.
 - Post-secondary category included the Teacher Training College, Nursing Training College, Polytechnic and the University levels).

CHAPTER FOUR

RESULTS

4.1 PARTICIPANT DEMOGRAPHIC CHARACTERISTICS

Data of the 38 peer-recruited women were included in the analysis of the demographic characteristics based on the fact that the same criteria of selection (they were from the same houses selected during the study and not more than three women in all, including the peer-recruited, from each of the selected houses were allowed to participate) and consenting processes were used. They were therefore expected to be similar to those recruited by the researchers. More importantly, the distributions of the demographic (Table 4.1) and risk characteristics of the 38 peer-recruited women were similar to those of the women who were recruited by the researchers; except for the distribution of religion (because almost all Muslims were within one community). Therefore, the potential bias due to the inclusion of the distribution of the 38 peer-recruited women was not evident. The table 4.1 shows the distribution of the demographic characteristics stratified by the reporting strategies in the study. Similar tables for the distribution of the risk characteristics stratified by reporting strategies were not reported. Therefore, the demographic characteristics of the 415 women (377 study recruited and 38 peer-recruited) who consented, and completed the study questionnaire are described in this section.

Table 4.1: Distribution of the demographic characteristics of the 415 recruited participants.

	Sub-categories	Reporting Strategy, n (%)				All Women
		Community based	Peer Recruited	Long duration	Short duration	
Grouped Age	15 - 19	6 (5.8)	2 (5.6)	12 (7.7)	6 (5.1)	26 (6.3)
	20 - 24	18 (17.5)	2 (5.6)	26 (16.8)	19 (16.1)	65 (15.8)
	25 - 29	28 (27.2)	8 (22.2)	36 (23.2)	25 (21.2)	97 (23.5)
	30 - 34	6 (5.8)	3 (8.3)	20 (12.9)	17 (14.4)	46 (11.2)
	35 - 39	12 (11.7)	7 (19.4)	15 (9.7)	18 (15.3)	52 (12.6)
	40 - 44	8 (7.8)	8 (22.2)	18 (11.6)	17 (14.4)	51 (12.4)
	45 - 49	6 (5.8)	2 (5.6)	15 (9.7)	7 (5.9)	30 (7.3)
	50 - 54	8 (7.8)	1 (2.8)	10 (6.5)	3 (2.5)	22 (5.3)
	55 - 59	4 (3.9)	3 (8.3)	2 (1.3)	4 (3.4)	13 (3.2)
	60 Or Older	7 (6.8)*	0 (0.0)	1 (0.6)	2 (1.7)	10 (2.4)
	Total	103 (100.0)	36 (100.0)	155 (100.0)	118 (100.0)	412 (100.0)
Religion	Christian	103 (100.0)	36 (100.0)	129 (82.7)*	105 (89.7)	373 (90.5)
	Muslim	0 (0.0)	0 (0.0)	25 (16.0)*	10 (8.5)	35 (8.5)
	Other	0 (0.0)	0 (0.0)	2 (1.3)	2 (1.7)	4 (1.0)
	Total	103 (100.0)	36 (100.0)	156 (100.0)	117 (100.0)	412 (100.0)
Marital Status	Unmarried	38 (38.0)	10 (28.6)	49 (31.8)	53 (44.9)	150 (36.9)
	Married	62 (62.0)	25 (71.4)	105 (68.2)	65 (55.1)	257 (63.1)
	Total	100 (100.0)	35 (100.0)	154 (100.0)	118 (100.0)	407 (100.0)
Educational Status	No Formal	21 (20.8)	6 (16.7)	30 (19.5)	11 (9.3)	68 (16.6)
	Primary	26 (25.7)	5 (13.9)	30 (19.5)	16 (13.6)	77 (18.8)
	Junior Secondary	39 (38.6)	14 (38.9)	69 (44.8)*	72 (61.0)*	194 (47.4)
	Senior Secondary	9 (8.9)	6 (16.7)	18 (11.7)	15 (12.7)	48 (11.7)
	Post-Secondary	6 (5.9)	5 (13.9)	7 (4.5)	4 (3.4)	22 (5.4)
	Total	101 (100.0)	36 (100.0)	154 (100.0)	118 (100.0)	409 (100.0)
Occupation	Unemployed	12 (12.0)	0 (0.0)	7 (4.7)	6 (5.2)	25 (6.2)
	Formally employed	18 (18.0)	9 (25.0)	23 (15.3)	16 (13.8)	66 (16.4)
	Skilled Worker	23 (23.0)	8 (22.2)	25 (16.7)	37 (31.9)	93 (23.1)
	Trader	34 (34.0)	16 (44.4)	75 (50.0)	47 (40.5)	172 (42.8)
	Agro-worker	13 (13.0)	3 (8.3)	20 (13.3)	10 (8.6)	46 (11.4)
	Total	100 (100.0)	36 (100.0)	150 (100.0)	116 (100.0)	402 (100.0)

* denotes for each sub-category of demographic characteristics, column proportions of a reporting strategy that differ significantly the other at the 95% confidence level. * The difference in religious characteristics that showed Muslim in only two of the four groups in the column was primary due to the fact that there was a major Muslim community that was approached at the early stage of the study.

The ages of the 415 participants ranged between 15 years and 70 years, and were not normally distributed; the test of normality (both Kolmogorov-Smirnov and Shapiro-Wilk) showed a significant difference ($p = 0.0001$) and a skewness of 0.68 ± 0.12 and Kurtosis of -0.16 ± 0.24 . The median age of the participants was 31.0 years with an interquartile range (IQR) of 16.0 years. The ages were subsequently grouped in a 5 year interval and the distribution presented in Table 4.1. The age group with the highest proportion of the participants was 25-29 years ($n = 97$; 23.5%), which was followed by the age group 20-24 years ($n = 65$; 15.8%). On the other hand the age groups with the lowest proportion of the women in this study were the age groups 60 years and older ($n = 10$; 2.4%) and 15-19 years ($n = 26$; 6.3%).

The distribution of the religious affiliations, were as follows, 90.5% Christians ($n = 373$), 8.5 % Muslims ($n = 35$) and 1.0% ($n = 4$) other religions. The Table 4.1 also shows that more than half (63.1%; $n = 257$) of the participants were married, while 36.9% were unmarried ($n = 150$). Among the women who were married, 79.4% (197 of 248) were in monogamous marriages while 14.9% ($n = 37$) were in polygamous marriages. The distribution of the educational status of the participants showed that 47.4% of the participants reported junior secondary as their highest attained educational level, as at the time of the study. The next most common educational status reported was the primary level, which was by 18.8% of the participants. The others were as follows, no formal education, 16.6%; senior secondary 11.7%; and post-secondary 5.4%. Additionally, 6.2% of the women were unemployed, and the occupations of those who were employed were categorised into four groups. Traders (42.8%) and skilled workers (23.1%) formed the majority of the participants. The others were in formal employment (16.4%) or in Agro-business (11.4%).

4.2 PARTICIPANTS REPORTING RATES/STATUS

4.2.1 Reporting strategy 1

Of the 473 eligible women invited to participate in this study, 415 consented and completed the study questionnaire. Of these, 377 participants were invited by the researchers while 38 were peer-invited. Since the number of women invited by their peers, of which 38 reported, could not have been known, and that they did not fit in any of the reporting strategies, these women were excluded from the analysis of the reporting rates as it will bias the estimated (over-estimate) overall reporting rate if included (figure 4.1).

Of the 377 participants invited by the researchers, 274 were invited to participate using reporting strategy 1 (which was the original strategy designed for the study). Among these, 118 of them opted to report within a week, while 156 opted to report at any time after a week but within 3 months after questionnaire administration, for specimen collection at the hospital (figure 4.1). These formed two sub-groups of reporting strategy 1, hereinafter referred to as short and long duration for reporting respectively. Among the 156 participants who opted for the long duration of reporting, 60 of them (38.5%) reported for specimen collection at the hospital within the stated period. Among the remaining 96 participants who did not report after the date they opted to, 27 of them were reached by a phone call, reminded of their participation and were allowed to set a new date. Of these, 12 participants (44.4% of the 27 participants or 12.5% of the 96 participants who did not report initially) reported for specimen collection at the hospital. On the other hand, among the 118 participants who opted for the short duration of reporting to the hospital, 55 (46.6%) reported within the duration they opted for.

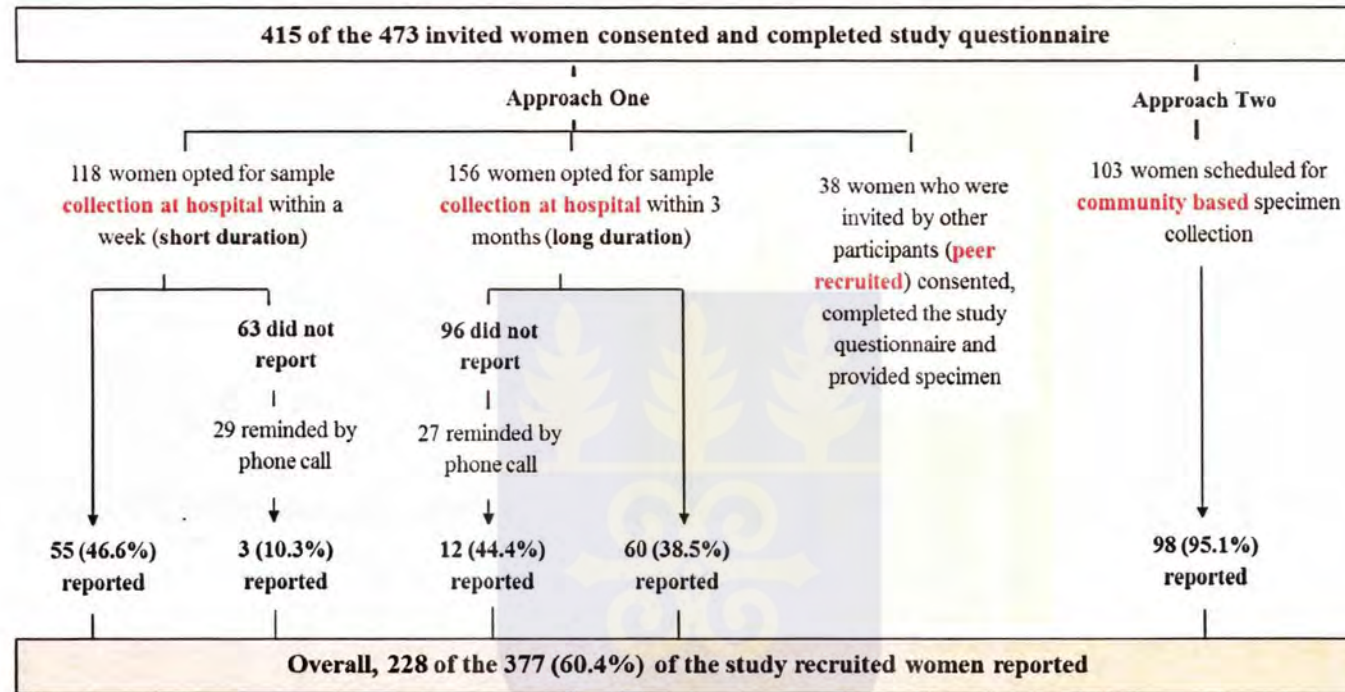


Figure 4. 1: Participant enrolment and response to specimen collection.

Among the remaining 63 participants who did not report, 29 were reached by a phone call, reminded of their participation and allowed to fix a new date. Of these 29 participants, only 2 (10.3% of those who were reached on their phone or 4.8% of those who did not report initially) reported to the hospital for specimen collection.

4.2.2 Reporting Strategy 2

In respect of the strategy that was developed on the field (reporting strategy 2), which involved specimen collection at a location within the communities, 103 women were invited to participate in this study by it and 98 (95.1%) of these women reported for specimen collection at the location within their respective communities (Figure 4.1).

4.2.3 Overall Reporting Rate

Overall, 228 of the 377 participants recruited by the study (excluding the 38 peer-recruited participants) reported for specimen collection; in other words, a reporting rate of 60.4% was achieved in this study. A comparison of the response rates for the three reporting strategies (long duration to report to the hospital, short duration to report to the hospital and report at a location within community), showed a statistically significant difference ($p = 0.0001$) and an LSD multiple comparison (post hoc analysis) indicated that all the pairwise comparisons of response rates were significantly different. The order therefore, from highest to lowest was, reporting at a location within a community (95.1%) > short duration to report (46.6%) > long duration to report (38.5%).

4.3 SEXUAL AND REPRODUCTIVE CHARACTERISTICS (KNOWN RISK FACTORS)

The sexual and reproductive characteristics of the 415 women (377 study recruited and 38 peer-recruited) who consented, and completed the study questionnaire were described together in this section. The inclusion of the data collected from the 38 peer-recruited participants was based on the reasons as described above for demographic characteristics. That is, the analysis of the risk characteristics stratified by the reporting strategies showed no significant differences.

4.3.1 Sexual Risk Characteristic (Risk Factors)

The ages at first sexual intercourse (AFSI) reported by the participants ranged between 9 years and 36 years and were not normally distributed; the test of normality (both Kolmogorov-Smirnov and Shapiro-Wilk) showed a significant difference ($p = 0.0001$), a skewness of 0.75 ± 0.12 and Kurtosis of 4.75 ± 0.24 . The median age at first sexual intercourse of the participants was 18 years with an interquartile range of 3.0 years. The ages were grouped into two sub-categories and the distribution depicted in Table 4.2. This table shows that slightly more than half of the participants (52.4%, $n = 208$) had their sexual debut before they were 19 years old. The remaining 47.6% started having sex when they were at the ages of 19 and older. A small number of the women ($n = 18$) did not state when they had their first sexual intercourse.

Regarding the number of male sexual partners the participants had had since they became sexually active (Table 4.2), a minimum of 1, a maximum of 10 with a median of 2 (interquartile range of 1) male sexual partners were recorded. Those who reported three or more male sexual

partners formed about 47.6% (n = 195) of the participants, while those who reported 2 and 1 male sexual partners were 27.6% and 22.2% respectively.

Table 4.2: Distribution of the categories of the participants' sexual risk factors

Sexual risk factors	Categories	Frequency	Percentage
Age at first sexual intercourse (years)	≤ 18	208	52.4
	≥ 19	189	47.6
	Total	397	
Sexual age (number of years since first sexual intercourse)	< 11	175	44.2
	11 – 19	97	24.5
	20 – 29	82	20.7
	≥ 30	42	10.6
	Total	396	
Lifetime number of male sexual partners (LNSP)	1	91	22.9
	2	111	28.0
	≥ 3	195	49.1
	Total	397	
Current number of male sexual partners (CNSP)	0	61	14.9
	1	347	84.6
	2	1	0.2
	3	1	0.2
	Total	410	
Use of condom during sexual intercourse	No	240	58.7
	Yes*	169	41.3
	Total	409	
Had an STI in the past 5-10 years	No	189	46.4
	Yes	218	53.6
	Total	407	
Other STIs	<i>Candida</i>	213	97.7
	<i>HIV</i>	1	0.5
	Gonorrhoea	2	1.0
	Total	216	

Italicized sub-categories are the high risk categories for HPV positivity.

** all were inconsistent users*

With respect to the current (at the time of the study) number of male sexual partners the participants had, most of them (84.6%) reported having one, while 14.9% reported they did not have a male sexual partner at the time of the study. Only one participant each reported 2 and 3 male sexual partners, while 5 participants did not report the number of male sexual partners they had. The table also shows slightly more than half, 58.7% ($n = 240$ of 409) of the participants stated that they and their male sexual partners had never used condoms during sexual intercourse, while 41.3% stated they and their male sexual partners had been using condoms, but inconsistently.

A little over half, 53.6% ($n = 218$) of the 407 participants who had indicated STI history, had contracted an STI in the past 5 to 10 years prior to this study, while 46.4% had not contracted any STI during that period and 8 women did not indicate their history of STI. Of these 218 participants who had contracted an STI, 97.7% ($n = 213$) reported they have been diagnosed of candidiasis, while only a single participant reported infection with the HIV, and 2 others reported a diagnosis of Gonorrhoea within the stated period.

4.3.2 Reproductive Risk Characteristics (Risk Factors)

The use of oral contraceptives (OC) was not common among the participants of this study (Table 4.3); only 23.3% ($n = 94$) of them stated they had or have been using OC within the previous 10 years at the time of the study. Most of them 76.7% ($n = 310$) reported they had not used any oral contraceptives within the stated period, while 11 women did not indicate their status of.

Table 4.3: Distributions of the reproductive risk factors among the participants.

Reproductive risk factors	Categories	Frequency	Percentage
Use of OC in the past 10 years	No	310	76.7
	Yes	94	23.3
	Total	404	100
Duration of OC use	< 1 year	47	52.2
	1 - 4 years	36	40.0
	≥ 5 years	7	7.7
	Total	90	100
History of menstrual difficulties	No	194	48.4
	Yes	207	51.6
	Total	401	100
Ever been pregnant	No	56	13.6
	Yes	355	86.4
	Total	411	100
Number of pregnancies (among ever pregnant)	1	57	16.1
	2 - 4	162	45.6
	≥ 5	136	38.3
	Total	355	
History of abortion (among ever pregnant)	No	182	51.5
	Yes	172	48.7
	Total	353	100
The number of induced abortion (among ever had an abortion)	1	100	58.1
	≥ 2	72	41.8
	Total	172	
History of miscarriage (among ever pregnant)	No	245	69.8
	Yes	106	30.2
	Total	351	100
The number of miscarriages (among ever had a miscarriage)	1	67	62.6
	≥ 2	39	36.4
	Total	106	100

Among the participants who had used OC, slightly more than half, 52.2% ($n = 47$ of 90), had used it for less than a year, 40.0% had used it for between 1 and 4 years and only 7.7%, of the

women had used OC for 5 or more years. The table 4.3 also shows that while about half of the participants, 51.6% ($n = 207$) had experienced menstrual difficulties, 48.4% ($n = 194$) stated they had not and 14 women did not provide any such information.

Also, a majority of the participants, 86.4% ($n = 355$ of 415) reported that they had ever been pregnant and the number of pregnancies per woman ranged between 1 and 18, with a median of 3.0 and an interquartile range of 4.0. A smaller proportion, 16.1% of these 355 participants had been pregnant only once, as at the time of the study. Those who had been pregnant for between 2 and 4 times constituted 45.6% ($n = 162$ of 355), while 38.3% had been pregnant for at least 5 times. One hundred and seventy two (48.7%) of the participants who had ever been pregnant, had ever had an abortion and 106 (30.2%) had ever experienced a miscarriage. The number of abortions reported ranged between 1 and 5 with a median of 1. More than half (58.1%) reported they had had one abortion while 41.8% reported they had had 2 or more abortions. The number of miscarriages reported, by the 106 participants who had experienced miscarriages, ranged between 1 and 5 with a median of 1. While 62.6% who miscarried had a single experience, 36.4% had miscarried two or more times.

4.3.3 Other Risk Characteristics (Factors)

While more than half (61.3%) of the 415 participants in this study were not consumers of alcoholic products as at the time of the study, 39.7% were. Almost all, 97.3%, of the 415 participants reported they had never smoked, with only 0.5% ($n = 1$) reported that she was a smoker, while 2.2% did not report their smoking status. Only a small proportion, 17.1% ($n = 71$) of the 415 participants had heard of cervical cancer; an even smaller proportion stated what, in their view, was the cause of cervical cancer (4.1%, $n = 17$) and how it could be prevented (1.7%,

n = 7). Of the 17 participants who stated a cause, 3 (17.6%) stated having sex at a tender age, 5 (29.4%) stated having multiple male sexual partners, 2 (11.7%) stated sexual transmission from males, 3 (17.6%) stated bacterial and virus infections of the cervix, 1 (5.8%) stated the insertion of dough in the vagina, 2 (11.7%) stated STI that when treated did not resolve and 1 (5.8%) stated unprotected sex, as the cause of cervical cancer. Of the 7 participants who stated a preventative measure, 1 (14.3%) stated being faithful to one sexual partner, 3 (42.8%) stated going for regular check-ups at the hospital and reporting unusual conditions to the doctor, 2 (28.6%) stated regular use of condom, 1 (14.3%) stated regular examination by cervical test, and 1 (14.3%) stated participating in screening activities, as preventative measures.

4.4 PARTICIPANT CHARACTERISTICS THAT INFLUENCED REPORTING AND CONDOM USE

4.4.1 Participant Characteristics that may have Influenced Reporting Status

These analyses of the association of the demographic characteristics with reporting status were performed separately for the three reporting strategies, which are; report within the community, long duration to report to the hospital and short duration to report to the hospital. This automatically excludes the 38 peer-recruited women. Among the participants who were to report within the community, it was determined that there were no statistically significant associations between reporting status with all the demographic characteristics (Table 4.4). Similarly, for the participants who opted for the short duration within which they were to report for specimen collection, reporting status was not significantly associated with any of the demographic characteristics (Table 4.5).

Table 4.4: Distribution and Association of participants' demographic characteristics with their reporting status, for Community specimen collection.

Demographic characteristics	Categories	Response to Community approach for specimen collection, n (%)			χ^2 p value
		Reported (n = 99)	Did not report (n = 4)	Total (N = 103)	
Age (categorised)	15 - 19	6 (6.1)	0 (0.0)	6 (5.8)	6.097 (0.730)
	20 - 24	16 (16.2)	2 (50.0)	18 (17.5)	
	25 - 29	27 (27.3)	1 (25.0)	28 (27.2)	
	30 - 34	6 (6.1)	0 (0.0)	6 (5.8)	
	35 - 39	12 (12.1)	0 (0.0)	12 (11.7)	
	40 - 44	7 (7.1)	1 (25.0)	8 (7.8)	
	45 - 49	6 (6.1)	0 (0.0)	6 (5.8)	
	50 - 54	8 (8.1)	0 (0.0)	8 (7.8)	
	55 - 59	4 (4.0)	0 (0.0)	4 (3.9)	
	60 or older	7 (7.1)	0 (0.0)	7 (6.8)	
	Total	99	4	103	
Marital status	Unmarried	36 (37.5)	2 (50.0)	38 (38.0)	Fisher exact (0.633)
	Married	60 (62.5)	2 (50.0)	62 (62.0)	
	Total	96	4	100	
Religion	Christian	99 (100)	4 (100)	103 (100)	0.001
	Muslim	0 (0.0)	0 (0.0)	0 (0.0)	
	Other	0 (0.0)	0 (0.0)	0 (0.0)	
	Total	99	4	103	
Educational status	No formal education	21 (21.6)	0 (0.0)	21 (20.8)	9.205 (0.056)
	Primary	25 (25.8)	1 (25.0)	26 (25.7)	
	Junior secondary	38 (39.2)	1 (25.0)	39 (38.6)	
	Senior secondary	7 (7.2)	2 (50.0)	9 (8.9)	
	Post-Secondary	6 (6.2)	0 (0.0)	6 (5.9)	
	Total	97	4	101	
Occupation	Unemployed	12 (12.5)	0 (0.0)	12 (12.0)	4.173 (0.383)
	Formal employment	17 (17.7)	1 (25.0)	18 (18.0)	
	Skilled worker	23 (24.0)	0 (0.0)	23 (23.0)	
	Trader	31 (32.3)	3 (75.0)	34 (34.0)	
	Agro-worker	13 (13.5)	0 (0.0)	13 (13.0)	
	Total	96	4	100	

Table 4.5: Distribution and Association of participant demographic characteristics with the reporting status, for Short duration hospital based specimen collection.

Variables	Categories	Response to Short appointment approach for specimen collection, n (%)			χ^2 p value
		Reported (n = 53)	Did not report (n = 37)	Total (N = 90)	
Age (categorised)	15 - 19	0 (0.0)	6 (16.2)	6 (6.7)	17.863 (0.037)*
	20 - 24	10 (18.9)	6 (16.2)	16 (17.8)	
	25 - 29	12 (22.6)	6 (16.2)	18 (20.0)	
	30 - 34	11 (20.8)	3 (8.1)	14 (15.6)	
	35 - 39	10 (18.9)	4 (10.8)	14 (15.6)	
	40 - 44	6 (11.3)	6 (16.2)	12 (13.3)	
	45 - 49	3 (5.7)	3 (8.1)	6 (6.7)	
	50 - 54	1 (1.9)	0 (0.0)	1 (1.1)	
	55 - 59	0 (0.0)	2 (5.4)	2 (2.2)	
	60 or older	0 (0.0)	1 (2.7)	1 (1.1)	
	Total	53	37	90	
Marital status	Unmarried	19 (35.8)	19 (51.4)	38 (42.2)	2.146 (0.143)
	Married	34 (64.2)	18 (48.6)	52 (57.8)	
	Total	53	37	90	
Religion	Christian	50 (94.3)	31 (86.1)	81 (91.0)	3.331 (0.189)
	Muslim	3 (5.7)	3 (8.3)	6 (6.7)	
	Other	0 (0.0)	2 (5.6)	2 (2.2)	
	Total	53	36	89	
Educational status	No formal education	6 (11.3)	5 (13.5)	11 (12.2)	2.406 (0.662)
	Primary	8 (15.1)	5 (13.5)	13 (14.4)	
	Junior secondary	31 (58.5)	24 (64.9)	55 (61.1)	
	Senior secondary	5 (9.4)	3 (8.1)	8 (8.9)	
	Post-Secondary	3 (5.7)	0 (0.0)	3 (3.3)	
	Total	53	37	90	
Occupation	Unemployed	3 (5.8)	2 (5.6)	5 (5.7)	4.046 (0.400)
	Formal employment	5 (9.6)	7 (19.4)	12 (13.6)	
	Skilled worker	20 (38.5)	10 (27.8)	30 (34.1)	
	Trader	21 (40.4)	12 (33.3)	33 (37.5)	
	Agro-worker	3 (5.8)	5 (13.9)	8 (9.1)	
	Total	52	36	88	

* indicates significant association

However, for the participants who opted for a long duration within which to report to the hospital for specimen collection, a statistically significant association was only obtained between age (continuous) and reporting status (Spearman Correlation = 0.202; $p = 0.022$). Those who reported, had a median age of 35.0 years, (IQR of 18.0 years), while those who did not report, had a median age of 29.0 years (IQR of 16.0 years). However, when the age was categorised it became statistically non-significant (Table 4.6). In other words, the reporting for specimen collection by each of the strategies was, to a large extent, random and not influenced by the demographic characteristics of the participants.

4.4.2. Participant Characteristics that may have influenced Condom Use

A chi-square analysis showed that there was a significant relationship between the use of condom and educational status, as well as between the use of condoms and marital status (Table 4.7; $p = 0.001$). It was determined that more participants than expected with educational status of junior secondary or higher stated the use of condom with their male sexual partners while less than expected participants with primary or no formal educational stated the use of condoms with their male sexual partners. Similarly, more of the unmarried participants than expected stated the use of condoms with their male partners while fewer of the married participants than expected stated same (Table 4.8). The association of condom use with the occupation of the participants was also significant ($p = 0.0001$) and revealed that for the unemployed, formally employed and skilled workers, more participants than expected, stated that they used condom while for the traders and agro-workers, less participants than expected reported the use of condoms during sexual intercourse.

Table 4.6: Distribution and Association of participants' demographic characteristics with their reporting status, for Long duration hospital based specimen collection.

Variables	Categories	Response to Long duration for specimen collection, n (%)			χ^2 p value
		Reported (n = 49)	Did not report (n = 80)	Total (N = 129)	
Age (categorised)	15 – 19	3 (6.1)	6 (7.6)	9 (7.0)	13.657 (0.135)
	20 – 24	6 (12.2)	16 (20.3)	22 (17.2)	
	25 – 29	9 (18.4)	19 (24.1)	28 (21.9)	
	30 – 34	5 (10.2)	12 (15.2)	17 (13.3)	
	35 – 39	6 (12.2)	7 (8.9)	13 (10.2)	
	40 – 44	8 (16.3)	5 (6.3)	13 (10.2)	
	45 – 49	3 (6.1)	10 (12.7)	13 (10.2)	
	50 – 54	6 (12.2)	4 (5.1)	10 (7.8)	
	55 – 59	2 (4.1)	0 (0.0)	2 (1.6)	
	60 or older	1 (2.0)	0 (0.0)	1 (0.8)	
	Total	49	79	128	
Marital status	Unmarried	11 (22.4)	27 (34.6)	38 (29.9)	2.124 (0.145)
	Married	38 (77.6)	51 (65.4)	89 (70.1)	
	Total	49	78	127	
Religion	Christian	44 (89.8)	64 (80.0)	108 (83.7)	2.672 (0.263)
	Muslim	5 (10.2)	14 (17.5)	19 (14.7)	
	Other	0 (0.0)	2 (2.5)	2 (1.6)	
	Total	49	80	129	
Educational status	No formal education	7 (14.3)	21 (26.9)	28 (22.0)	4.576 (0.334)
	Primary	13 (26.5)	14 (17.9)	27 (21.3)	
	Junior secondary	23 (46.9)	34 (43.6)	57 (44.9)	
	Senior secondary	5 (10.2)	5 (6.4)	10 (7.9)	
	Post-Secondary	1 (2.0)	4 (5.1)	5 (3.9)	
	Total	49	78	127	
	Unemployed	3 (6.8)	2 (2.5)	5 (4.1)	5.717 (0.221)
Occupation	Formal employment	5 (11.4)	12 (15.2)	17 (13.8)	
	Skilled worker	7 (15.9)	12 (15.2)	19 (15.4)	
	Trader	26 (59.1)	37 (46.8)	63 (51.2)	
	Agro-worker	3 (6.8)	16 (20.3)	19 (15.4)	
	Total	44	79	123	

Table 4.7: Association between use of condom and demographic characteristics of the participants

Demographic Characteristics	Category	Use of condom					
		Observed (Expected)				Association, (p-value)	
		No	%	Yes	%	Spearman, R	χ^2
Age	15 – 19	12 (14.6)	5.0	13 (10.4)	7.7	-0.39 (<0.001)	52.05 (0.001)
	20 – 24	28 (36.9)	11.7	35 (26.1)	20.7*		
	25 – 29	38 (56.8)	15.9	59 (40.2)	34.9*		
	30 – 34	24 (26.9)	10.0	22 (19.1)	13.0		
	35 – 39	36 (29.9)	15.1	15 (21.1)	8.9		
	40 – 44	40 (29.9)	16.7*	11 (21.1)	6.5		
	45 – 49	22 (17.6)	9.2	8 (12.4)	4.7		
	50 – 54	19 (12.9)	7.9*	3 (9.1)	1.8		
	55 – 59	10 (7.6)	4.2	3 (5.4)	1.8		
	60 or Older	10 (5.9)	4.2*	0 (4.1)	0.0		
Educational status	No Formal Education	57 (39.8)	24.1*	11 (28.2)	6.5	-	30.27 (0.001)
	Primary	51 (45.1)	21.5	26 (31.9)	15.5		
	Junior Secondary	100 (113.5)	42.2*	94 (80.5)	56.0		
	Senior Secondary	21 (25.7)	8.9	23 (18.3)	13.7		
	Post-Secondary	8 (12.9)	3.4	14 (9.1)	8.3*		
Marital status	Unmarried	65 (86.4)	27.4	82 (60.6)	49.4*	-	20.33 (0.001)
	Married	172 (150.6)	72.6*	84 (105.4)	50.6		
Occupation	Unemployed	13 (14.5)	5.6	12 (10.5)	7.2	-	26.89 (0.001)
	Formal Employment	25 (37.2)	10.8	39 (26.8)	23.4*		
	Skilled Worker	50 (54.1)	21.6	43 (38.9)	25.7		
	Trader	104 (99.4)	44.8	67 (71.6)	40.1		
	Agro-Worker	40 (26.7)	17.2*	6 (19.3)	3.6		

* The proportion in the Yes category was significantly different from that of the No category

Table 4.8: Sexual risk characteristics of the participants' which are associated with the use of condom

Sexual risk Characteristics	Category	Use of condom				χ^2 , (p-value)
		Observed (Expected)				
		No	%	Yes	%	
Age at first sexual intercourse	< 20	136 (141.6)	60.4	112 (106.4)	66.3	1.33 (0.515)
	>= 20	89 (83.4)	39.6	57 (62.6)	33.7	
Sexual age	< 11	66 (98.6)	29.5	107* (74.4)	63.3	51.30 (0.001)
	11-19	60 (54.7)	26.8	36 (41.3)	21.3	
	20 – 29	63 (46.7)	28.1	19* (35.3)	11.2	
	> 29	35 (23.9)	15.6	7* (18.1)	4.1	
	0	41 (35.7)	17.2	20 (25.3)	11.8	
Current number of male partners	1	196 (201.2)	82.4	148 (142.8)	87.6	Fisher's exact test's (0.159)
	2	0 (1.0)	0.0	1 (0.0)	0.6	
	3	1 (1.0)	0.4	0 (0.0)	0.0	
Lifetime number of sexual partners	1	60 (51.9)	26.4	30 (38.1)*	18.0	4.3 (0.116)
	2	63 (63.4)	27.8	47 (46.6)	28.1	
	>= 3	104 (111.8)	45.8	90 (82.2)	53.9	
Had any STI within the past 10 years	No	126 (110.6)	53.2*	63 (78.4)	37.5	9.692 (0.002)
	Yes	111 (126.4)	46.8	105 (89.6)	62.5*	
Ever had an abortion	No	149 (138.2)	62.3	87 (97.75)	51.5	4.791 (0.029)
	Yes	90 (100.75)	37.7	82 (71.245)	48.5*	
Number of abortion	1	55 (52.84)	61.1	46 (48.15)	56.1	0.445 (0.505)
	>= 2	35 (37.15)	38.9	36 (33.848)	43.9	

* The proportion in the Yes category was significantly different from that of the No category

Age, analysed as a numerical variable, was significantly but inversely associated with condom use (Spearman correlation $R = -0.39$; $p = 0.001$). Age remained significant after it was categorized ($p = 0.001$) and showed that more participants than expected within the ages of 15

years to 34 years stated the use of condom. Also, there were significant associations (Table 4.7) between the use of condoms and the sexual-age (as a numerical variable) of the participants as well as with the lifetime number of male sexual partners (numerical variable). The use of condoms decreased among the participants with higher sexual age, while there was an increase in the stated use of condoms among the participants with a higher lifetime number of male sexual partners.

However, the association between condom use and the following (analysed as numerical variables) were all not statistically significant: the AFSI ($p > 0.515$), and current number of male sexual partners ($p > 0.159$). These variables were categorized and the association with condom use were re-analysed by chi-square (Table 4.8). The sexual age remained significantly associated with the state of condom use ($\chi^2 = 51.30, p = 0.001$). However, the lifetime number of sexual partners became statistically not significantly associated ($\chi^2 = 4.3, p = 0.116$) with the stated use of condom (Table 4.8). The AFSI after being categorized as such (<19, 19 – 21, and >21 years) remained not significantly associated ($p = 0.515$) with the stated use of condom. Also, after the current number of male sexual partners were categorized into a dichotomous variable (currently have or do not have a male sexual partner) there was not a significant association (2-sided Fisher's exact test's $p = 0.159$) with the stated use of condom.

4.5 ADDITIONAL FINDINGS - SPECIMEN COLLECTION

4.5.1 Pre-performance preference of Specimen Collection Method

Since the preference for specimen collection were not in any way related to the reporting strategies, data of all the 253 participants (including the 38 peer recruited) who reported for specimen collection were used in this analysis.

Of this number, 226 participants provided specimen by self and health personnel collection, 7 participants opted for only health personnel collection and 18 participants opted for only self-collection. SC specimen of 2 women and HPC specimens of two other women were lost (Figure 4.2). The major reasons stated by the participants' pre-performance preference for only health-personal specimen collection included blindness (one participant) and the feeling that they might hurt themselves with the self-specimen collection brush. On the other hand, the major reasons for a pre-performance preference for only self-specimen collection were that the participants did not want the insertion of the speculum, some were just afraid of the speculum while others resisted the speculum insertion.

4.5.2 Post-performance Preferences for Specimen Collection Method

Out of the 253 participants who provided at least one specimen, about a half, 50.3% ($n = 127$) preferred the health personnel specimen collection after performing the sample collection, while 20.2% ($n = 51$) preferred self-specimen collection after performing (Table 4.9). The others, either did not have any post-performance preference (19.4%, $n = 49$) or did not state their post-performance preference (9.9%, $n = 24$). The commonest reason for preferring self-specimen collection post-performance was the experience of slight pain and/or fear for the health personnel specimen collection method (Table 4.10). This was stated by 68.6% ($n = 35$) of participants who

preferred self-specimen collection post-performance. Six other participants (11.8%), each preferred self-specimen collection post-performance because of the privacy it provided and how simple it was to perform.

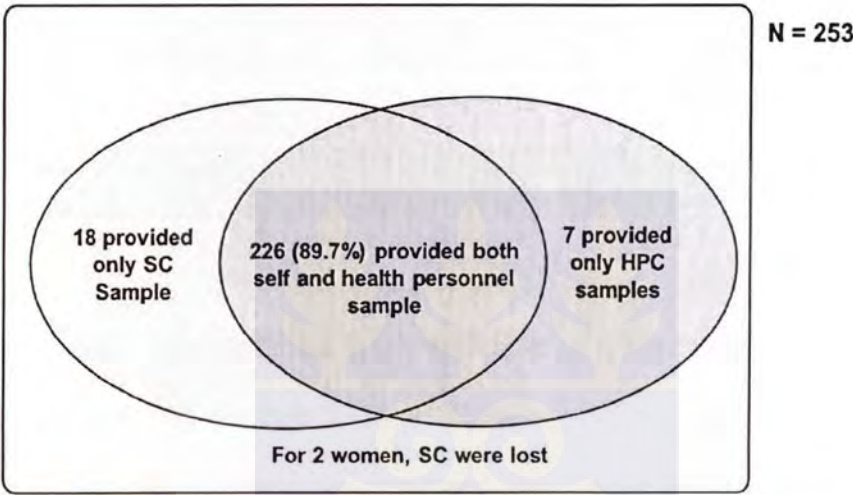


Figure 4.2: Distribution of the performance of sample collection.

Table 4.9: Distribution of preferences for specimen collection methods after providing the specimen.

Preference	Frequency	Percentage
Self-specimen collection	51	20.2
Health personnel specimen collection	127	50.3
I have no preference	49	19.4
Not reported	25	9.9
Total	252	100.0

Table 4.10: Reasons for the preference of self-specimen collection.

Reasons	Frequency	Percentage
The health personnel sample collection was with slight pain and /or fear	35	68.6
Issues of privacy	6	11.8
The self - specimen collection was simple and easy to perform	6	11.8
Can't explain	1	2.0
Stated no reason	3	5.9
Total	51	100.0

Of the 127 participants who preferred health personnel specimen collection, the commonest reason, stated by 85 (66.9%), was that the health personnel specimen collection was most likely better performed than the self-specimen collection they performed, because the nurse and medical officers were professionals, experienced and knew what they were about (Table 4.11).

Sixteen participants (12.6%) stated that since they had no knowledge of cervical cancer screening, the health personnel specimen collection was preferable. The third most common reason, stated by 11.0% (n = 14) of the participants, was that they were not confident they performed the self-specimen collection correctly. Other reasons given for the preference of health personnel specimen collection by a few of the participants are shown in Table 4.11.

Table 4.11: Reasons for the preference of health personnel specimen collection.

Reasons	Frequency	Percentage
The health personnel would have done them better because they were professionals, experienced and know what they were about	85	66.9
The participants had no idea about cervical screening	16	12.6
The participants were not confident they performed the self-specimen collection correctly.	14	11.0
In order to obtain good and/or the right results	2	1.4
The health personnel would find another problem or unusual occurrences if any around the sampling area	2	1.4
The Pap smear was not difficult and there was no pain	1	0.7
The participant was afraid she was going to hurt herself with the self-specimen collection device	1	0.7
The participant stated that the self-sampling device is not user-friendly	1	0.7
The nurse could see what she was doing, but the participant did not see what she did during self-specimen.	1	0.7
Had no problem with the health personnel examinations and sample collection	1	0.7
Gave no reason	6	4.7
Total	127	100.0

4.6 HUMAN PAPILLOMAVIRUS (HPV) TESTING

4.6.1 HPV Genotype Specific Prevalence

The quality of the specimens obtained from the 253 women who provided specimens for this study were tested by a real-time PCR amplification of the human housekeeping gene, RNase H. Although 17 of the 244 self-collected and 6 of the 233 health personnel collected specimen were not consistently positive with triplicate analyses of the RNase H gene (2 of 3 or 1 of 3 were positive), they were included in the HPV analysis. For the determination of the agreement

between the two specimen collection methods, only data generated by the 226 women who provided both specimens were used.

Out of the 46 HPV genotypes tested for, 37 and 26 genotypes were detected with the self and the health personnel collected specimens respectively (Table 4.12). Overall, 38 genotypes were detected with both methods of specimen collection. The 8 genotypes that were not detected in this study were HPV11, HPV13, HPV32, HPV61, HPV71, HPV89, HPV97 and HPV26. Only HPV43 was not detected with SC specimen, but was detected with HPC specimen. Also, HPV33, HPV44, HPV53, HPV68, HPV69, HPV70, HPV72, HPV73, HPV85, HPV86, HPV90 and HPV91 were detected with SC specimens but not with HPC specimens. The six most detected HPV genotypes with the self-collected specimen were HPV16 (5.9%; 95% CI 3.0% – 9.0%), HPV35 (4.7%; 95% CI 2.0% – 8.0%), HPV40 (4.7%; 95% CI 2.0% – 8.0%), HPV45 (4.3%; 95% CI 2.0% – 7.0%), HPV58 (4.0%; 95% CI 2.0% – 7.0%) and HPV18 (3.6%; 95% CI 1.0 – 6.0) (Table 4.12).

On the other hand, the six most detected HPV genotypes with the health personnel collected specimen were HPV35 (2.8%; 95% CI 1.0% – 5.0%), HPV58 (2.8%; 95% CI 1.0% – 5.0%), HPV16 (2.4%; 95% CI 1.0% – 5.0%), HPV18 (2.4%; 95% CI 1.0% – 5.0%), HPV56 (2.4%; 95% CI 1.0% – 5.0%) and HPV45 (2.0%; 95% CI 0.0% – 4.0%). For each of the detected genotypes, a higher prevalence was detected with the self-collected sample than was detected with the health personnel collected specimen (Table 4.12), however, the differences were not significant (p values were between 0.092 and 0.743).

Table 4.12: HPV genotype prevalence determined with each specimen collection method.

HPV Genotype	Self-Collected Specimen, n=244			Health Personnel Collected Specimen, n = 230		
	n	Prevalence	95% CI	n	Prevalence	95% CI
HPV16	15	5.9	3.0 – 9.0	6	2.4	1.0 – 5.0
HPV35	12	4.7	2.0 – 8.0	7	2.8	1.0 – 5.0
HPV40	12	4.7	2.0 – 8.0	4	1.6	0.0 – 4.0
HPV45	11	4.3	2.0 – 7.0	5	2.0	0.0 – 4.0
HPV58	10	4.0	2.0 – 7.0	7	2.8	1.0 – 5.0
HPV18	9	3.6	1.0 – 6.0	6	2.4	1.0 – 5.0
HPV66	8	3.2	1.0 – 6.0	6	2.4	1.0 – 5.0
HPV59	7	2.8	1.0 – 5.0	3	1.2	0.0 – 3.0
HPV6	7	2.8	1.0 – 5.0	3	1.2	0.0 – 3.0
HPV31	7	2.8	1.0 – 5.0	2	0.8	0.0 – 2.0
HPV74	7	2.8	1.0 – 5.0	1	0.4	0.0 – 1.0
HPV42	5	2.0	0.0 – 4.0	5	2.0	0.0 – 4.0
HPV52	5	2.0	0.0 – 4.0	4	1.6	0.0 – 4.0
HPV51	5	2.0	0.0 – 4.0	3	1.2	0.0 – 3.0
HPV62	5	2.0	0.0 – 4.0	2	0.8	0.0 – 2.0
HPV81	5	2.0	0.0 – 4.0	1	0.4	0.0 – 1.0
HPV83	5	2.0	0.0 – 4.0	1	0.4	0.0 – 1.0
HPV72	5	2.0	0.0 – 4.0		ND	
HPV90	5	2.0	0.0 – 4.0		ND	
HPV67	4	1.6	0.0 – 3.0	1	0.4	0.0 – 1.0
HPV53	4	1.6	0.0 – 3.0		ND	
HPV56	3	1.2	0.0 – 3.0	1	0.4	0.0 – 1.0
HPV33	3	1.2	0.0 – 3.0		ND	
HPV70	3	1.2	0.0 – 3.0		ND	
HPV87	2	0.8	0.0 – 2.0	2	0.8	0.0 – 2.0
HPV39	2	0.8	0.0 – 2.0	1	0.4	0.0 – 1.0
HPV30	2	0.8	0.0 – 2.0	1	0.4	0.0 – 1.0
HPV54	2	0.8	0.0 – 2.0	1	0.4	0.0 – 1.0
HPV44	2	0.8	0.0 – 2.0		ND	
HPV86	2	0.8	0.0 – 2.0		ND	
HPV82	1	0.4	0.0 – 1.0	1	0.4	0.0 – 1.0
HPV84	1	0.4	0.0 – 1.0	1	0.4	0.0 – 1.0
HPV68	1	0.4	0.0 – 1.0		ND	
HPV69	1	0.4	0.0 – 1.0		ND	
HPV85	1	0.4	0.0 – 1.0		ND	
HPV73	1	0.4	0.0 – 1.0		ND	
HPV43		ND		1	0.4	0.0 – 1.0

ND = not detected; order of listing was, according to HPV prevalence determined by self-collected samples

4.6.2 Overall and Risk Type HPV Prevalence

The overall prevalence of HPV infection as determined by the 244 *self-collected specimen* was 43.1% (95% CI 38.0% – 51.0%) while that determined by the 233 *health-personnel collected specimen* was 23.3% (95% CI of 19.0% – 31.0%). The difference between these prevalences was significant ($\chi^2 = 28.943$; $p = 0.0001$; Table 4.13).

The prevalence of each risk type group was determined with the 244 self-collected specimens and the 233 health personnel collected specimen separately; the prevalence of high risk (HR) HPV types (see section 2.8.1 for all the genotypes that make each risk HPV group) detected with self-collected specimen was 27.2% (95% CI of 23.0% – 34.0%) and that detected with the health-personnel collected specimen was 16.6% (95% CI of 14.0% – 24.0%). Similarly, the prevalence of low risk (LR) HPV was 23.3% (95% CI of 19.0% – 30.0%) with self-collected specimen and 9.9% (95% CI of 6.0% – 14.0%) with health-personnel collected specimen (Table 4.13). On the other hand, the prevalence of the probable high risk (PHR) HPV types were very low, 4.7% (95% CI of 2.0% – 8.0%) with self-collected specimen and 0.4% (95% CI of 0.0% – 1.0%) with health personnel collected specimen.

Single HPV genotype infections were detected among 62 (56.9%) of the 109 women who were HPV positive with self-collected specimen while 43.1% of them were infected with multiple genotypes of HPV. On the other hand, while single HPV infections were detected among 43 (72.1%) of the 59 women who were HPV positive with their health-personnel collected specimen. 27.1% of health-personnel collected specimens were infected with multiple genotypes of HPV. A chi-square test of the difference in the proportions of the following; overall HPV

infections, single/multiple, HR, and LR HPV infections determined by the two methods were each significant (Table 4.13).

4.6.3 Agreement between Self and Health Personnel Collected Specimen

A Cohen's kappa (k) analysis for each of the HPV infection category (Table 4.13) using data on the 226 women who provided both SC and HPC showed a significant level of agreement ($p = 0.0001$) in the detections of HPV with self and health-personnel collected specimen. However, the level of agreement between the two specimen collection methods was poor for both the detection of PHR HPV infections and for the detection single HPV infections (kappa values, $k = 0.147$ and 0.128 respectively). The agreements between the two collection methods were fair for the detection of LR HPV infection, the overall HPV infection and multiple HPV infection, ($k = 0.328$, 0.321 and 0.286 respectively). The agreement between the two specimen collection methods for HR HPV infections was moderate ($k = 0.402$).

As indicated in table 4.14, of the 226 participants who provided both self and health personnel collected specimen, 44 (19.5%) were HPV positive with both their HPC and SC specimens; and 108 (47.8%) were HPV negative with both their HPC and SC specimens. Therefore, 152 (44 + 108) of the 226 participants had the same results in respect of overall HPV detection by both SC and HPC, resulting in an overall HPV detection concordance between SC and HPC specimen of 67.2%. Furthermore, 13 (5.8%) of the 226 participants were HPV positive by their HPC specimen, but HPV negative by their self-collected specimen. Conversely, 61 (27.0%) of the 226 participants were HPV positive by their self-collected specimen but HPV negative by their HPC specimen. Therefore, the discordance in the overall HPV detection between HPC and SC

specimen was 32.8%. A McNemar's test indicated that the marginal proportions of the overall HPV detection were all significantly different in each case ($p = 0.0001$).

Table 4.13: HPV prevalences and the extent of agreements between self and health personnel collected specimen.

Infection Categories	Self-collection		Health Personnel-collection		Cohen's Kappa,	
	Prevalence	95% CI	Prevalence	95% CI	k	p value
Overall HPV	43.1	38.0 – 51.0	23.3	19.0 – 31.0	0.321	0.0001
HR HPV	27.2	23.0 – 34.0	16.6	14.0 – 24.0	0.402	0.0001
LR HPV	23.3	19.0 – 30.0	9.9	6.0 – 14.0	0.328	0.0001
PHR HPV	4.7	2.0 – 8.0	0.4	0.0 – 1.0	0.147	0.0001
	Proportion ^a		Proportion ^b			
Single	56.9%		72.9%		0.128	0.048
Multiple infection	43.1%		27.1%		0.286	0.0001

HR = high risk; LR = low risk; PHR = probable high risk. Prevalence was determined with 244 SC specimens and 230 HPC specimens. Chi-square and Kappa analysis were performed with 266 pairs of SC and HPC specimen. ^a of the 109 HPV positive with SC; ^b of the 59 HPV positive with HPC

Table 4.14: Concordance and discordance in the detection of HPV infections with self and health personnel collected specimen

HPV status	Self-collected specimen	Health personnel collected specimen		Total	Concordance	Discordance	McNemar's test (p value)
		Negative	Positive				
Overall HPV	Negative	108 (47.8%)	13 (5.8%)	121	(108 + 44) 67.3%	(13 + 61) 32.8%	0.0001
	Positive	61 (27.0%)	44 (19.5%)	105			
	Total	169	57	226			
HR HPV	Negative	147 (65.2%)	13 (5.7%)	160	(148 + 29) 77.9%	(13 + 37) 22.1%	0.0001
	Positive	37 (16.3%)	29 (12.8%)	66			
	Total	184	42	226			

% are of the overall total of participants who performed both methods, n = 226.

A similar analysis of the concordance and discordance in the detection of high risk (HR) HPV by the SC and HPC specimen was performed. Of the 226 participants who provided both SC and HPC specimen, 148 participants were HR HPV positive by their SC and HPC specimen, while 29 participants were HR HPV negative by both their SC and HPC specimens. Therefore, the concordance of the two specimen types in the detection of HR HPV was 77.9%. On the other hand, while 13 (5.7%) of participants were HR HPV positive by their HPC specimen they were HR HPV negative by their SC specimen. Additionally, 37 (16.3%) of the 226 participants were HR HPV negative by their HPC specimen, but they were HR HPV positive by their SC specimen. Therefore, the discordance between the two types of specimen in respect of the detection of HR HPV was 22.1%. A McNemar's test indicated that the marginal proportions of the high risk HPV detection were all significantly different in each case ($p = 0.0001$).

4.6.4 Test of Hypothesis on HPV Prevalence

A comparison of the overall prevalence of HPV by the HPC specimen (23.3%; 95% CI 19.0% - 31.0%) with the hypothesized 26.3% prevalence (Thomas *et al.*, 2004) was by a Binomial Test at 95% confidence level. The p-value obtained by this test was 0.310 and since the p-value was more than 0.05, the study failed to reject the null hypothesis (H_0). Therefore, the HPV prevalence of the Akuse sub-district of the Eastern Region of Ghana as determined with the HPC specimen was not different from the HPV prevalence of 26.3% reported by Thomas *et al.*, 2004.

4.6.5 Age Specific Prevalence (ASP)

The overall HPV, high risk HPV and low risk HPV age specific prevalence (ASP) were determined for both self and health personnel collected specimen (Figures 4.3 – 4.5). Age was determined to be significantly associated with overall HPV prevalence ($\chi^2 = 36.1$; $p = 0.001$), HR HPV prevalence ($\chi^2 = 26.09$; $p = 0.002$) and with LR HPV prevalence ($\chi^2 = 21.49$; $p = 0.011$). Additionally, the ASP determined with SC specimen were usually higher than those determined with HPC specimen.

The ASP of overall HPV infections determined with the self-collected specimen was trimodal with peaks at 20-24 years, 40-44 years and 55-59 years. In respect of the HPC specimen, the ASP of the overall HPV infection was bimodal with peaks at 20-24 years and 55-59 years. For both SC and HPC specimen, the overall HPV prevalence was the same for the age group 45-49 years and the age group of > 59 years. The ASP of high risk HPV prevalence (Figure 4.4) was similar to that of the overall HPV prevalence described above. That is, for the SC specimen a trimodal pattern was obtained with peaks at 20-24 years, 40-44 years and 55-59 years. For the

HPC specimen, the age-specific HR HPV prevalence pattern was bimodal with peaks at 20-24 years and 55-59 years.

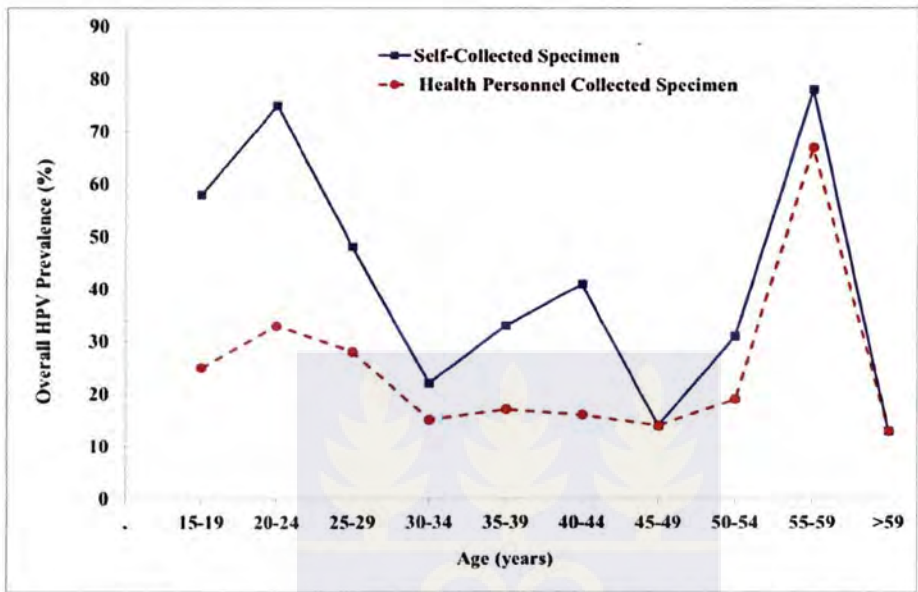


Figure 4.3: Age specific overall HPV infection prevalence obtained with self and health personnel collected specimen.

The HR HPV prevalence for the SC and HPC specimen were the same for women 45 years and older (Figure 4.4). The age-specific prevalence of low risk (LR) HPV determined with SC was bimodal with peaks at 20-24 years and 55-59 years while that for the HPC specimen was also bimodal but with peaks at 15-19 years and 55-59 years (Figure 4.5).

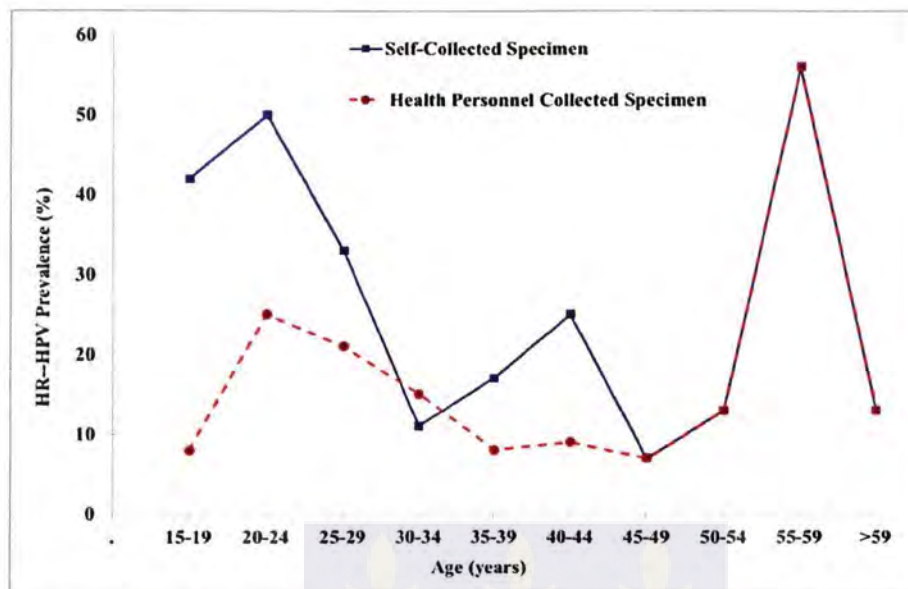


Figure 4.4: Age specific high risk (HR) HPV infection prevalence obtained with self and health personnel collected specimen.

4.7 TEST OF ASSOCIATIONS BETWEEN SEXUAL RISK CHARACTERISTICS AND HPV POSITIVITY

Although the study reported in this thesis was not powered to determine and quantify the association between the sexual risk behaviour characteristics and HPV positivity the crude association between these was useful for comparing the two specimen collection methods as to which of the two was more informative (informativeness was defined as obtaining the appropriate directionality of the association, as has been widely reported/excepted for each of the risk characteristics). Therefore, multivariable analyses to obtain adjusted odds ratios were not performed, which would have been misleading.

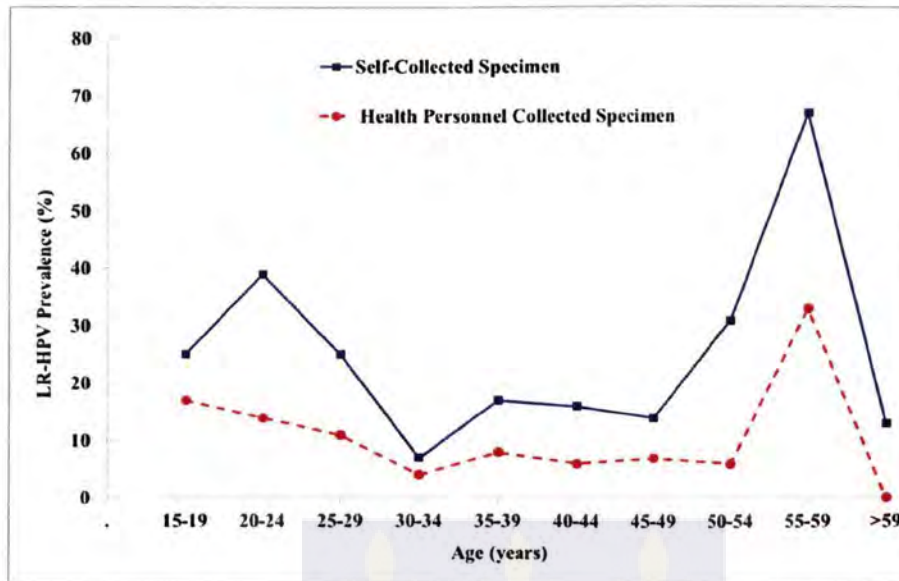


Figure 4.5: Age specific low risk (LR) HPV infection prevalence obtained with self and health personnel collected specimen.

4.7.1 Univariable Association between Sexual Risk Characteristics and HPV Infection Positivity Determined With Self-Collected Specimen

The odds ratios obtained with the univariable logistic regression analysis for the association between the sexual characteristics and HPV positivity determined with self-collected specimens were shown in Table 4.15. The AFSI, when analysed as a numerical variable, recorded an odds ratio of less than 1.00 for its association with overall HPV positivity, HR HPV positivity, single infection and multiple infection and an odds ratio of 1.03 (95% CI 0.94 - 1.12) for its association with LR HPV positivity; however, all the odds ratio were not statistically significant. The analysis was repeated with AFSI categorized into two groups, younger than 20 years and 20 years or older. With the former as the reference group, it was determined that an AFSI of 20 years or older recorded an odds ratio of less than 1.00 for its association with overall HPV

positivity, HR HPV positivity, single infection and multiple infection while the odds ratio for its association with LR positivity was greater than 1.00. Except for the odds ratio for the association with HR HPV positivity which was 0.46 (95% CI 0.24-0.85), all the others were not significant (Table 4.15).

Compared to currently (as at the time of the study) not having a male sexual partner, the odds ratio for the association between having sexual partner and each of the HPV infection positivity was less than 1.00 and not statistically significant (Table 4.15). Similarly, the odds ratio recorded for the association between the lifetime number of male sexual partners (LNSP) and each category of HPV positivity were all not significant; however, the odds ratios were greater than 1.00 except for single HPV infection. Categorising LNSP into three groups (1, 2 and ≥ 3), with having 1 LNSP as the reference group, revealed an odds ratio of greater 1.00 for the association between having 2 LNSP and each of the categories of HPV positivity except for LR HPV positivity [OR = 0.98 (95% CI 0.79 - 1.22)]. On the other hand, the odds ratio for the association between having 3 or more LNSP and Overall, LR and single HPV positivity were less than 1.00. All these odds ratios were not significant.

When analysed as a numerical variable, sexual-age was significantly associated with the overall HPV positivity (OR = 0.97, 95% CI 0.94 – 0.99) and with single HPV infection positivity (OR = 0.96; 95% CI 0.93 – 0.99). However, it was not significantly associated with HR, LR and multiple HPV positivity, which also recorded odds ratios of less than 1.00 (Table 4.15).

Table 4.15: Crude odds ratio for the association between sexual characteristics and HPV infection positivity determined with self-collected specimen

Risk Factor	Category	Odds ratio (95% CI), with Self-Collected Specimen				
		Any HPV	HR HPV	LR HPV	Single Infection	Multiple Infection
Age at first sexual intercourse		0.95 (0.88 - 1.03)	0.89* (0.80 - 0.98)	1.03 (0.94 - 1.12)	0.95 (0.87 - 1.04)	0.95 (0.86 - 1.05)
Age at first sexual intercourse (categorised)	< 20	Reference				
	≥ 20	0.64 (0.37 - 1.09)	0.46* (0.24 - 0.85)	1.37 (0.75 - 2.49)	0.67 (0.36 - 1.27)	0.60 (0.29 - 1.22)
Currently have a male sexual partner	No	Reference				
	Yes	0.80 (0.38 - 1.67)	0.62 (0.29 - 1.35)	0.80 (0.35 - 1.84)	0.92 (0.37 - 2.29)	0.67 (0.27 - 1.68)
Lifetime number of sexual partners		1.04 (0.87 - 1.25)	1.07 (0.88 - 1.30)	1.01 (0.82 - 1.24)	0.98 (0.79 - 1.22)	1.14 (0.90 - 1.45)
Lifetime number of sexual partners (categorised)	1	Reference				
	2	1.04 (0.50 - 2.15)	1.27 (0.57 - 2.85)	0.78 (0.34 - 1.81)	1.03 (0.44 - 2.38)	1.06 (0.40 - 2.82)
	≥ 3	0.87 (0.45 - 1.71)	1.09 (0.51 - 2.31)	0.96 (0.45 - 2.04)	0.76 (0.35 - 1.67)	1.05 (0.43 - 2.56)
Sexual-age		0.97* (0.94 - 0.99)	0.97 (0.95 - 1.00)	0.99 (0.97 - 1.02)	0.96* (0.93 - 0.99)	0.97 (0.94 - 1.00)
Sexual-age (categorised)	< 11	Reference				
	11-19	0.35* (0.18 - 0.67)	0.36* (0.17 - 0.76)	0.27* (0.11 - 0.66)	0.46* (0.21 - 0.99)	0.22* (0.08 - 0.58)
	20 - 29	0.31* (0.15 - 0.63)	0.38* (0.17 - 0.84)	0.32* (0.13 - 0.78)	0.44* (0.20 - 0.98)	0.16* (0.05 - 0.50)
	> 29	0.54 (0.23 - 1.27)	0.65 (0.26 - 1.63)	1.38 (0.58 - 3.29)	0.34 (0.10 - 1.13)	0.75 (0.28 - 2.01)
Contracted STI	No	Reference				
	Yes	1.33 (0.79 - 2.22)	1.34 (0.76 - 2.3)	1.10 (0.61 - 2.00)	1.24 (0.68 - 2.29)	1.45 (0.73 - 2.86)
Condom use	No	Reference				
	Yes	1.44 (0.86 - 2.41)	1.09 (0.62 - 1.92)	1.48 (0.82 - 2.67)	1.11 (0.60 - 2.06)	2.03* (1.04 - 3.98)

* the odds of the association was significant, since the range of 95% CI excludes 1.0.

Repeating the analysis with sexual age categorized into the following groups, <11 years, 11-19 years, 20-29 years and >29 years, with < 11 years as the reference group, it was determined that having a sexual age of between 11 and 19 years as well as between 20 and 29 years were significantly associated with each of the categories of the HPV positivity categories, the odds ratios were all less than 1.00 (Table 4.15).

Compared to having not contracted an STI in the past 10 years, having contracted an STI was not significantly associated with each of the categories of the HPV positivity with odds ratios greater than 1.00. Similar findings were determined for the use of condom. Compared to not using a condom, the odds ratio for the association of condom use with each of the categories of HPV positivity was greater than 1.00 and not statistically significant (Table 4.15).

4.7.2 Univariable Association between Sexual Characteristics and HPV Infection Positivity Determined with Health Personnel-Collected Specimen

The odds ratios obtained by the univariable logistic regression analysis for the association between the sexual characteristics and HPV positivity determined with health personnel-collected specimens were shown in Table 4.16. The AFSI when analysed as a numerical variable recorded odds ratio of less than 1.00 for its association with overall HPV positivity, LR HPV positivity, and multiple infection positivity, while it recorded OR of 1.00 (95% CI 0.91-1.10) and 1.02 (95% CI 0.93-1.12) for its association with single and HR HPV infection positivity respectively. However, these associations were not statistically significant.

Table 4.16: Crude odds ratio for the association between sexual characteristics and HPV infection positivity determined with health personnel-collected specimen

Sexual characteristics	Category	Health Personnel-Collected Specimen, OR (95% CI)				
		Any HPV	HR HPV	LR HPV	Single Infection	Multiple Infection
Age at first sexual intercourse		0.99 (0.91-1.08)	1.02 (0.93-1.12)	0.93 (0.81-1.07)	1.00 (0.91-1.10)	0.96 (0.82-1.14)
Age at first sexual intercourse (categorised)	< 20	Reference				
	≥ 20	1.15 (0.62-2.11)	1.16 (0.59-2.31)	1.00 (0.42-2.39)	1.19 (0.59-2.36)	1.05 (0.36-3.01)
Currently have a male sexual partner	No	Reference				
	Yes	0.64 (0.29-1.41)	0.79 (0.32-1.96)	0.62 (0.22-1.79)	0.48 (0.21-1.11)	2.19 (0.28-17.37)
Lifetime number of sexual partners	LNSP	1.01 (0.83-1.25)	1.03 (0.82-1.29)	1.03 (0.78-1.37)	0.97 (0.77-1.23)	1.12 (0.81-1.55)
Lifetime number of sexual partners (categorised)	1	Reference				
	2	1.00 (0.43-2.35)	0.72 (0.28-1.86)	1.24 (0.34-4.50)	1.02 (0.39-2.64)	0.94 (0.20-4.46)
	≥ 3	1.05 (0.48-2.28)	0.88 (0.38-2.04)	1.44 (0.45-4.68)	0.96 (0.40-2.32)	1.30 (0.33-5.09)
Sexual-age		1.00 (0.97-1.02)	0.99 (0.96-1.02)	1.00 (0.96-1.04)	1.00 (0.97-1.03)	0.98 (0.93-1.03)
Sexual-age (categorised)	< 11	Reference				
	11-19	0.49 (0.22-1.07)	0.62 (0.27-1.46)	0.32 (0.09-1.17)	0.62 (0.26-1.48)	0.25 (0.05-1.18)
	20 - 29	0.38* (0.15-0.95)	0.33 (0.11-1.02)	0.42 (0.11-1.54)	0.51 (0.19-1.37)	0.15 (0.02-1.24)
	> 29	1.13 (0.45-2.81)	1.27 (0.47-3.42)	1.09 (0.33-3.65)	1.36 (0.49-3.74)	0.71 (0.14-3.49)
STI	No	Reference				
	Yes	1.14 (0.63-2.08)	1.15 (0.58-2.27)	0.97 (0.42-2.24)	1.32 (0.66-2.62)	0.78 (0.28-2.18)
Use condom	No	Reference				
	Yes	1.15 (0.63-2.09)	1.08 (0.55-2.12)	1.35 (0.59-3.11)	0.95 (0.48-1.89)	1.87 (0.67-5.27)

* the odds of the association was significant, since the range of 95% CI excludes 1.0.

These associations were determined again with AFSI categorized into two groups, younger than 20 years and 20 years or older, with the former as the reference group. It was determined that an AFSI of ≥ 20 years as compared to at an AFSI of < 20 years recorded odds ratio slightly above 1.00 for each category of HPV infection positivity.

Compared to currently (as at the time of the study) not having a male sexual partner, the odds ratio for the association between having a male sexual partner and each of the HPV infection positivity was less than 1.00 except for multiple infection positivity, which was 2.19 (85% CI 0.28-17.37); however all these OR were not statistically significant. Also, the odds ratios determined for the association between the LNSP and each category of the HPV positivity were slightly greater than 1.00 except for single HPV infection positivity, which recorded an OR of 0.97 (95% CI 0.77-1.23); but were all not statistically significant. With having 1 LNSP as the reference group, the odds ratios determined for the association of having 2 LNSP with HR and multiple HPV positivity were both less than 1.00 while those with overall, LR and single HPV infection positivity were slightly greater than 1.00. On the other hand, the odds ratio for the association of having 3 or more LNSP with overall, LR and multiple HPV positivity were greater than 1.00. All these odds ratios for the associations with LNSP were all not significant.

When analysed as a continuous variable, the odds ratio for the associations of the sexual age of the participants with their HPV infection positivity were not significant, with odds ratios approximately equal to 1.00. Repeating the analysis with sexual age as a categorized variable, with < 11 years as the reference group, it was determined that the odds ratio for the association of having a sexual age of between 11 and 19 years as well as having a sexual age between 20 and 29 years with each category of HPV infection positivity were less than 1.00 and not statistically

significant. However, having a sexual age of more than 29 years recoded odds ratio of greater 1.00 for its association with each category of HPV infection positivity except for the association with multiple HPV infection positivity. Compared to not having an STI, contracting an STI in the past 10 years was not significantly associated with overall (OR = 1.14; 95% CI 0.63-2.08), HR (OR = 1.15; 95% CI 0.58-2.27 and single infection HPV positivity (OR = 1.32; 95% CI 0.66-2.62). With respect to the use of the condom, it was determined that compared to not using condoms, the odds ratio for the association of condom use with each of the categories of HPV positivity was greater than 1.00 except for single HPV infection positivity but all were not statistically significant.

4.8 HPV VARIANT ANALYSES

4.8.1 HPV16 Variants

Each of the 21 specimen determined by genotyping to be HPV16 positive, gave positive results for the LCR HPV16 type specific PCR for sequencing. However, three (3) of the sequences were too short (did not include nucleotides within most of the important variable regions) for further analysis and therefore were excluded in the nucleotide alignment and phylogenetic tree constructed. The sequences of 19 HPV16 isolates were aligned with the region between positions 7469 and 7840 of the complete genome of the HPV16 prototype NC0015626.2 (Kennedy *et al.*, 1991; Seedorf *et al.*, 1985). However, the sequences of 2 of the 19 HPV16 isolates (isolate-866 and isolate-628) were shorter by 13 nucleotides (due to incomplete sequencing) and aligned to position 7826 of the prototype. The alignment also included sequences of selected HPV16

variants obtained by an NCBI BLAST of the isolates' sequences. These variants were also included in the phylogenetic tree constructed (Figure 4.6).

Nucleotide sequence variations were observed in 30 positions within the LCR positions 7460 – 7840. Of these 30 positions, 11 were observed exclusively with the HPV16 isolates obtained in this study and were located with the positions 7801 to 7828 (Table 4.17). Specifically, the nucleotide positions 7804 and 7807 showed most of the exclusive variation among the study isolates; with three isolates each for each variant position. A close look at the overall nucleotide sequence variations showed that for the following isolates, (of this study) isolate-937, isolate-866 and isolate-618, the observed variations were similar to those of the HPV16 Africa type 1 variants; specifically, the variation in the sequence of isolate-937 were similar to that of the HPV16 variant BF325_Af-1 (100% coverage and id of 99%) with an additional transversion of an A to T at position 7836. On the other hand, isolate-866 was similar to the HPV16 variants KF466576_AFR1a and KF466592_AFR1b (99% coverage and id of 100%) but showed transversions of a T to G at position 7824 and a G to T at position 7825. Isolate-618 was similar to the HPV16 variants KF466576_AFR1a and KF466592_AFR1b (100% coverage and id of 99%) but showed a transversion of a C to A at position 7801, a T to G at position 7804, and a transition of an A to G at positions 7807 and 7809.

Also, the sequence variation of the isolates; isolate-500, isolate-612, and isolate-617 were similar to that of BF236_Af-2 (100% coverage and id of 99%) but with the following additional variations (Table 4.17); a transition of G to A at position 7815 for isolate-500; another transition of an A to G at position 7807 for isolate-612 and a transversion of T to G at position 7804 as well as another transversion of C to A at position 7828 for isolate-617. The nucleotide sequence

variations of the isolate-628 were similar to that of the HPV16 variant KF466652._AFR2b (100% coverage and id of 99%) but with the following additional variation, a transversion of a T to G at positions 7804 and 7812, and a transition of an A to G at positions 7807 and 7808. The nucleotide sequence of isolate-999 matched exactly with that of the HPV16 variant KF466626_Af-2a (100% coverage and id of 100%). Also, the nucleotide sequences variations of isolate-759, isolate-514 and isolate-511 were matched exactly with that of the HPV16 variant Rw862_Af-2. Similarly, the nucleotide sequences of the following isolates 765, 609, 606, 603, 602, 601, 569, 517 and 607 matched exactly with that of BF236_Af-2 (100% coverage and id of 100%). In all, 15% of the isolate were determined to be HPV16 African type 1 variants, all of which had additional nucleotide variations not observed in the prototype and other African type 1 variants included in the alignment. Five percent of the isolates were HPV16 African type 2a variants, while 80% of the isolates were the African type 2b variants, 25% of which had additional nucleotide variations not observed in both the prototype and other African type 2b variants included in the alignment.



Table 4.17 Nucleotide sequence variations between positions 7469 and 7840 (within the long control region (LCR)) of HPV16 variants.

HPV16 Isolates	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7		
	4	4	4	5	5	5	6	6	6	7	7	7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	8	8	8	8		
	8	8	8	0	2	6	1	6	8	0	1	2	2	4	6	8	8	0	0	0	0	0	0	1	1	2	2	2	3	3	3	
	1	4	8	6	0	9	0	8	8	2	3	8	9	2	3	0	5	1	4	6	7	8	9	2	5	4	5	8	3	6	7	
NC001526_E1	A	A	G	A	G	T	A	C	C	G	T	A	A	T	C	T	C	C	T	T	A	A	A	T	G	T	G	C	G	A	A	
SiHa cells	.	.	.	.	A	.	.	.	.	.	.	.	.	.	-	-	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
HPV16	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
W0122_E2	.	.	.	.	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Qv02706_EA	.	.	.	.	A	.	.	.	.	C	.	.	C	.	G	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Rw677_AA1	.	C	A	.	A	.	.	T	A	.	.	C	.	G	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Qv03545_AA2	G	C	A	G	A	.	.	T	A	.	.	C	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
KF466576_Afla	.	.	A	.	A	.	.	.	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	
KF466592_Aflb	.	.	A	.	A	.	.	.	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	
Rw918_Af-1	.	.	A	.	A	.	.	.	A	.	A	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	
BF325_Af-1	.	.	A	.	A	G	.	.	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	T	.	.	
937	.	.	A	.	A	G	.	.	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	.	T	T	.	
866	.	.	A	.	A	.	.	.	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	G	T	-	-	-	-	
618_2	.	.	A	.	A	.	.	.	A	.	.	.	.	.	T	.	T	A	G	.	G	.	G	.	G	.	.	.	T	.	.	
618	.	.	A	.	A	.	.	.	A	.	.	.	.	.	T	.	T	A	G	.	G	.	G	.	G	.	.	.	T	.	.	
KF466626_Af-2a	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	C	.	.	.	.	.	.	.	A	.	T	C	G
999	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	C	.	.	.	.	.	.	.	A	.	T	C	G
Rw862_Af-2	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	A	.	T	C	G
759	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	A	.	T	C	G
514	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	A	.	T	C	G
511	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	.	.	.	A	.	T	C	G

table 17. continued

	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
HPV16 Isolates	4	4	4	5	5	5	6	6	6	7	7	7	7	7	7	7	8	8	8	8	8	8	8	8	8	8	8	8
	8	8	8	0	2	6	1	6	8	0	1	2	2	4	6	8	8	0	0	0	0	0	0	1	1	2	2	3
	1	4	8	6	0	9	0	8	8	2	3	8	9	2	3	0	5	1	4	6	7	8	9	2	5	4	5	8
NC001526_E1	A	A	G	A	G	T	A	C	C	G	T	A	A	T	C	T	C	C	T	T	A	A	A	T	G	T	G	C
KF466652_AFR2b	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	T	C
628	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	G	.	G	G	.	G	.	.	A	-
BF236_Af-2	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
765	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
609	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
606	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
603	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
602	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
601	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
569	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
517	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
607	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	.	A	.	.	C
800	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	.	.	.	.	A	.	A	.	C
612	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	.	G	.	.	.	.	A	.	.	C
617	.	C	A	.	A	.	.	T	A	.	.	.	.	.	T	.	T	.	G	.	.	.	.	.	A	A	.	C

☒ Variation exclusive to isolates of this study.
 ☒ Variations within sequences of known HPV16 variants
 ☐ Same nucleotide as reference isolate
 ☐ deletion of nucleotide at that position

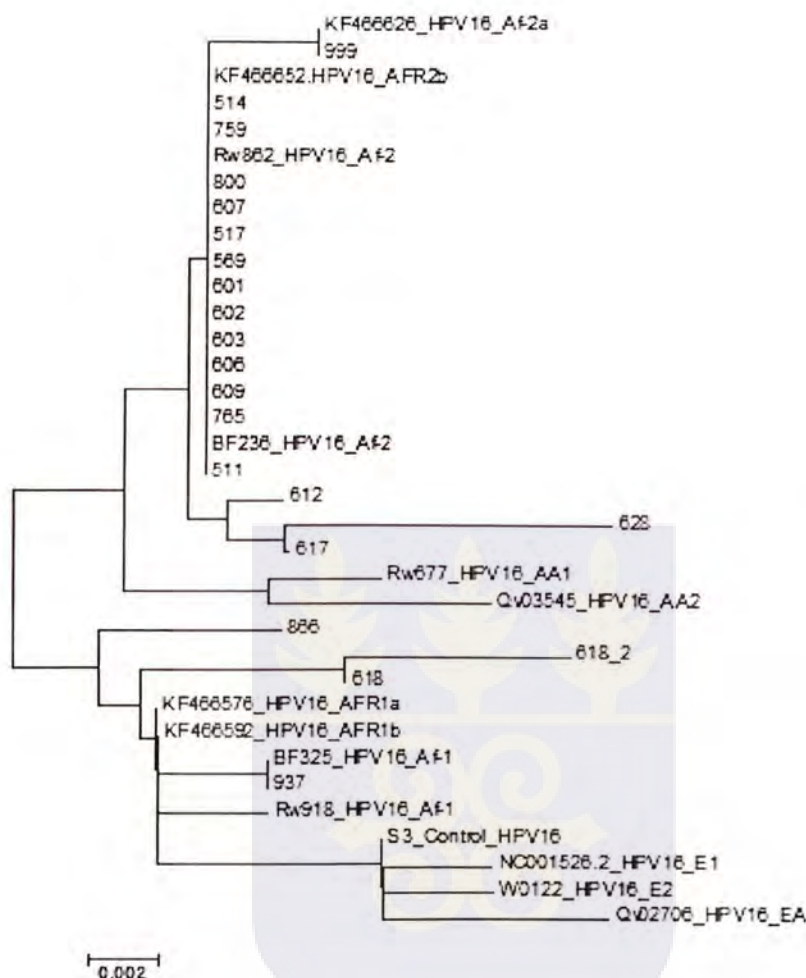


Figure 4.6 An evolutionary relationship of HPV16 isolates based on sequences between the genome positions 7469 and 7840 within the long control region (LCR.).

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.04170288 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 876 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.*, 2011).

4.8.2 HPV18 Variants

Of the 15 specimen detected as HPV18 positive by genotyping, 13 were positive by an LCR HPV18 type specific PCR for sequencing. However, one of these 13 gave a poor sequence and therefore was excluded in the alignment and phylogenetic tree constructed. The sequences obtained with the other 12 isolates were aligned with the region between positions 7464 and 7839 of the complete genome of the HPV18 Prototype AY262282_A1 (Narechania *et al.*, 2005). Also included in the alignment were sequences of selected HPV18 variants, obtained by an NCBI BLAST of the isolates' sequences, which were also included in the phylogenetic tree construction (Figure. 4.7).

Nucleotide variations were determined in 35 positions, 13 of which were exclusively observed with the sequences of the study isolates. The positions that showed most of the exclusive variation among the isolates in this study were 7813 and 7799. Position 7813 revealed variation in 5 isolates, 3 of which were a change to an A and the other 2 were deletions. Position 7799 on the other hand revealed variation in 3 isolates, 2 of which were changes to an A and the other one was a deletion (Table 4.18).

Specifically, isolate-858 matched exactly with the HPV18 prototype AY262282_A1 (Narechania *et al.*, 2005) with no variations in all positions aligned. However, isolate-613 showed similar sequence variation as those of HPV18 A3 variant, Qv26861 but with additional variation, which were a transition of a C to T at position 7551, a transition of a G to A at position 7563 and a transversion of T to G at position 7833. The sequence variations of isolate-917 were similar to those of the HPV18 B1 variant Rw750 (100% coverage and id of 99%) but showed additional variations; transversion of C to G at position 7768, transition of a G to A at positions 7799 and

7813 and a transition of a T to C change at position 7814. A comparison of the nucleotide variation of the isolate-561, isolate-679, isolate-553 and isolate-809 revealed an exact match with the nucleotide variations determined for the HPV18 B2 variant, BF309. However, for the isolate-591, although it showed a similar nucleotide variation to that of the HPV18 B2 variant BF309 (100% coverage and id of 99%), an additional transition of a G to A at position 7813 was determined. Also, isolate-572 showed the following additional variations compared to that of the HPV18 B2 variant BF309; deletions at positions 7799 and 7813.

The isolates 851 and 555 showed similar nucleotide variations with that of the HPV18 B3 variant BF380 (100% coverage and id of 99%); however, isolate-851 revealed an additional transversion of a C to A at position 7795, a transition of a G to A at position 7799 and a deletion at 7813, while isolate-555 revealed a deletion at position 7529. Isolate-627 revealed a nucleotide variation similar to that of the HPV18 C variant Qv39775 (100% coverage and 98% identify) but with the following additional variations; a transition of a T to C at position 7765, a transversion of T to G at position 7769, a transition of an A to G at position 7770, a transition of a C to T at positions 7771 and 7773, a transversion of a T to A at position 7774, a transversion of C to G at position 7800 and a deletion at position 7813 (Table 4.18).

Table 4.18 Nucleotide sequence variations between positions 7464 and 7839 (within the long control region (LCR)) of HPV18 variants.

HPV18 isolates	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
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☒ Variation exclusive to isolates of this study.
 ☐ variations within sequences of known HPV18 variants
 ☐ Same nucleotide as reference isolate
☐ deletion of nucleotide at that position

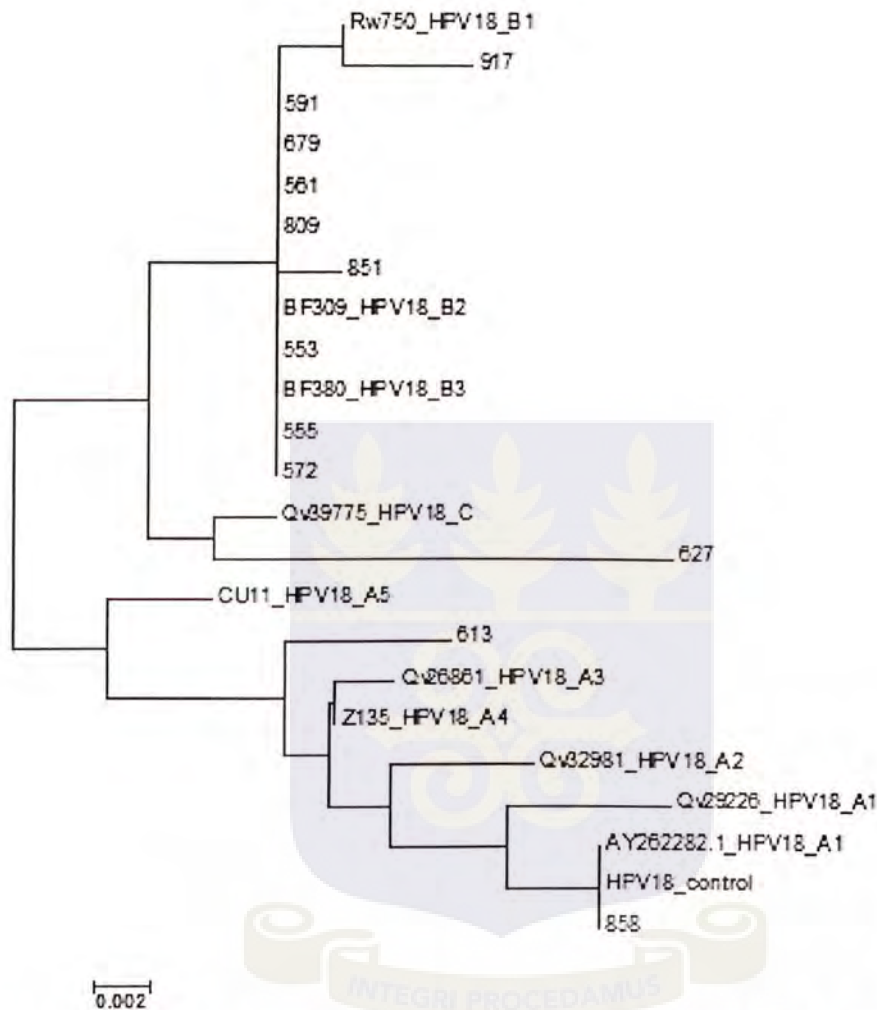


Figure 4.7 An evolutionary relationship of the taxa of HPV18 isolates based on sequences between the genome positions 7464 and 7839 within the long control region (LCR).

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.04170288 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 876 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.*, 2011).

Table 4.19 Nucleotide sequence variations between positions 7074 and 7858 (within the long control region (LCR)) of HPV45 variants.

[illegible]

■ variations exclusive to isolates of this study. ■ variations within sequences of known HPV45 variants

☐ Same nucleotide as reference isolate ☐ deletion of nucleotide at that position

4.8.3 HPV45 Variants

Of the 18 specimen that were determined to be HPV45 positive by genotyping, 13 gave amplification products with a three-primer overlapping LCR HPV45 specific PCR for sequencing. The sequences of 4 of the 13 isolates were determined to have been too short, therefore were excluded in the alignment and construction of the phylogenetic tree. The other 9 sequences were aligned with the genome of the HPV45 prototype X74479.1 (Deliuss and Hofmann, 1994) from position 7074 through 7858/1 to 102. Also included in the alignment were sequences of selected HPV45 variants obtained by an NCBI BLAST of the isolates' sequences, which were also included in the phylogenetic tree construction (Figure 4.8).

Nucleotide sequence variations were determined in 35 positions, 2 of which were observed exclusively with the sequences of the study isolates. Position 97 showed the most variations among the isolates in this study; in six isolates 460, 583, 632, 831, 880, 1025 with the nucleotide change of A to G as compared to the prototype HPV45 variant. Isolate-820 matched exactly with the prototype HPV45 variant while isolate 512 revealed a nucleotide variation of a transition of a C to T at position 7114. The isolate-444 revealed a similar nucleotide variation with the HPV45 A1 variant, Qv20214, but with a transition of an A to G at position 97 (Table 4.19).

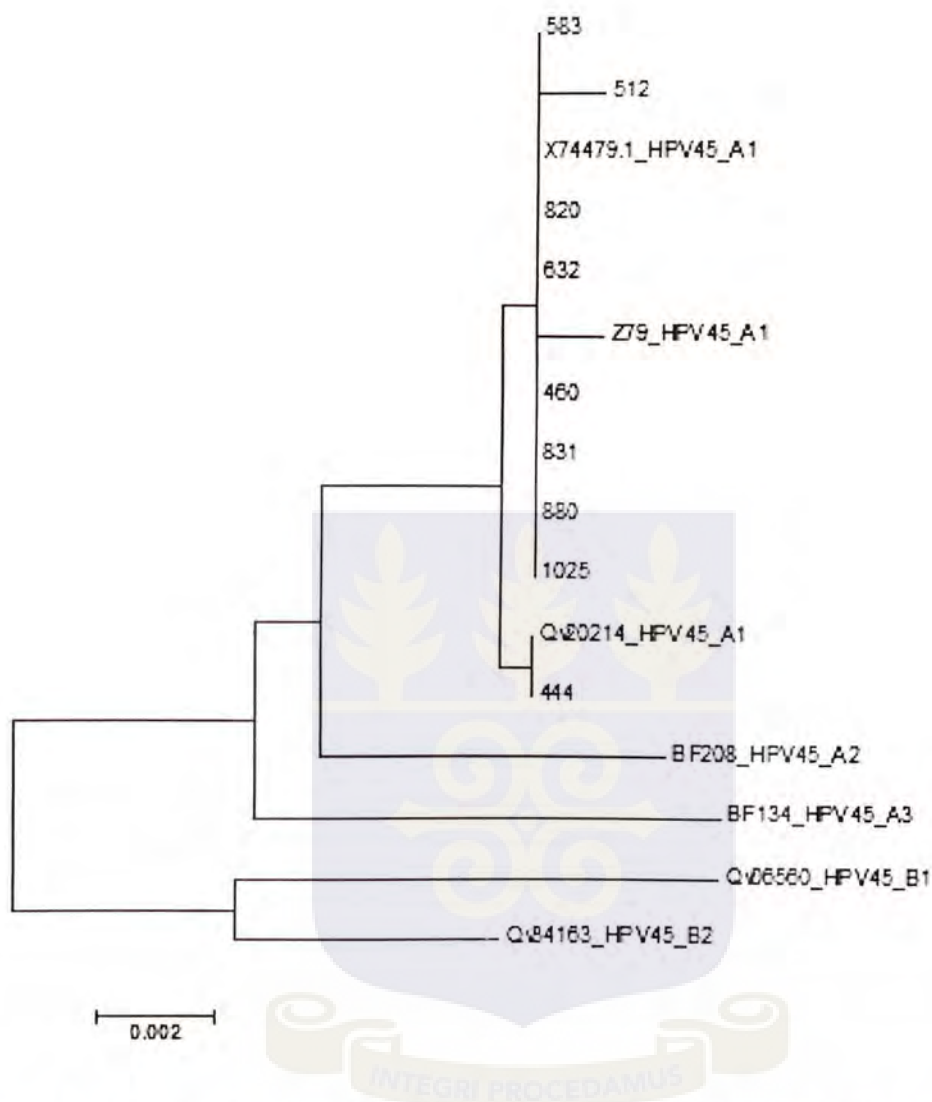


Figure 4.8 An evolutionary relationship of HPV45 isolates based on sequences between the genome positions 7074 and 7858 within the long control region (LCR.).

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The optimal tree with the sum of branch length = 0.04170288 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. The analysis involved 16 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 876 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.*, 2011).

4.9 CERVICAL LESIONS AND ABNORMALITIES

Of the 251 microscopic slides prepared and evaluated for malignant lesions, 212 (84.4%) had satisfactory smears while 39 (15.6%) had unsatisfactory smears for evaluation (Table 4.20). There was no statistically significant association between the adequacy of the smear and the site or location of specimen collection and smear preparation (Table 4.20).

Table 4.20: Distribution and association between the location of specimen collection and the adequacy of the smears for evaluation

Specimen collection point	Adequacy of smear, n(%)			Chi-Square (p value)
	Unsatisfactory	Satisfactory	Total	
Hospital	28 (18.4)	124 (81.6)	152 (60.4)	2.092 (0.148)
Community	11 (11.1)	88 (88.9)	99 (39.6)	
Total	39 (15.5)	212 (84.5)	251	

All the satisfactory smears evaluated (n = 212) were negative for cervical malignant lesion and showed no dyskaryosis and intraepithelial lesion. However, 19.5% (n = 41) of them were diagnosed with other conditions, the commonest of which was Candida infections (Table 4.21).

Table 4.21: Distribution of diagnosis based on Pap smear evaluation

Diagnosis	Frequency	Percentage
Dyskaryosis	0	0.0
Acute vaginitis	4	2.1
Atrophic cervicitis	9	4.2
Bacterial vaginosis	15	7.4
Candida infection	13	6.3
No Abnormality detected	170	80.0
Total	212	100.0



CHAPTER FIVE

DISCUSSION

5.1 PARTICIPANT CHARACTERISTICS

5.1.1 Distributions demographic Characteristics

Although the data from the 38 peer-recruited women were potentially going to bias the findings of the study, an analysis of the demographic, reproductive and sexual characteristics of the 38 women indicated that they were not different from the women recruited by the study (Table 4.1). Also, when these data were stratified by the reporting strategies, the exclusion or inclusion of the 38 women did not make any statistical difference. Therefore, demographic, reproductive and sexual characteristics of the 38 women were included in the analysis of the overall distributions of the demographic, reproductive and sexual characteristics, although the distribution for each strategy was also indicated. However, these 38 women were excluded from the analysis of each of the individual reporting strategies since these women did not report by any of them (Figure 4.1). That is, they did not opt for a reporting time for which it was to be assessed whether they reported on time or not.

Therefore, considering the distribution of the demographic data, the finding that the distribution of the religious status of the participants was not uniform among the different reporting strategies (Table 4.1) is explainable by the following. These are that there was only one community, known as the Zongo community, where almost all the Muslims in the sub-district were resident. This was the first community the study engaged its participants, and with the initial approach of the

study, which recorded a low response rate. No community was approached with more than one strategy and therefore, the Zongo community was not approached with any of the other strategies. Therefore, the difference in the distribution of the religious status of the women was a reflection of the inherent non-uniform distribution of Muslims in the sub-district.

5.1.2 Distribution of Sexual Risk Characteristics

As indicated in sections 1.3 and 2.5, for a relative small cross-sectional study, as the one reported in this thesis, the distribution of the categories of the known risk factors were used as indicators of the potential of detecting an HPV infection, since the association of these characteristics and HPV infection among the participants in this study could not be appropriately assessed due to the relatively small sample size (this study was not powered to determine these associations). Therefore for this study, the question was whether the Akuse sub-district could be said to be at high risk for HPV infection, based on the distribution of the sub-categories of each of the known sexual risk factors for HPV infection. If so, will this high risk status be confirmed by the prevalence of HPV infection determined in this study? This initial supposition that the Akuse sub-district was a high at risk community for the acquisition of HPV infection (section 3.1-3.2) was confirmed by the findings of the study as has been presented in table 4.2. That is, as indicated in section 2.5.1.2, AFSI of ≤ 18 years is high risk for HPV infection (Erickson *et al.*, 2013; Luhn *et al.*, 2013; Bahmanyar *et al.*, 2012; The International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Berrington de González *et al.*, 2004) and since slightly more than half of the participants of this study, 52.4%, were within this category, the sub-district was likely to have slightly more women who are infected with HPV. That is, it was a moderate at risk community for HPV infection. Similarly, and as indicated in section

2.5.1.2, the sexual age (the difference between the current age and AFSI) characteristic's categories of < 11 years and between 11 years and 19 years are high risks for HPV positivity (Bahmanyar *et al.*, 2012; Plummer *et al.*, 2012; Lenselink *et al.*, 2008). Therefore, the finding that 68.7% of the participants of this study were within these sub-categories (Table 4.2) suggested that most of the women in the sub-district were more likely to be HPV positive. In other words the sub-district was a moderate to high at risk community for HPV infection. Furthermore, the finding that almost half (49.1%) of the women in this study were within the ≥ 3 sub-category of lifetime number of male sexual partners, the high risk sub-category for HPV positivity (Erickson *et al.*, 2013; Luhn *et al.*, 2013; Bahmanyar *et al.*, 2012; Almonte *et al.*, 2011; International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Shields *et al.*, 2004), further suggest that the sub-district was a moderate at risk community for HPV positivity. This suggested moderate to high risk for HPV infection among the women in this sub-district was further supported by the findings that all the women were not consistent users of condom although 41.3% stated they had ever used condom. This was so because, it is the consistent use of condom that protects against HPV infection (Burchell *et al.*, 2006; Winer *et al.*, 2006), as indicated in section 2.5.1.4. Additionally, the finding that 53.6% of the women had had an STI infection within the past 5-10 years and that 97.7% of them were infected with Candida provide further supportive evidence for a high potential of HPV infection among the women, since a history of STI and an infection with Candida are high risk for HPV positivity (Burchell *et al.*, 2006; Samoff, 2005). Overall, the distribution of the categories of sexual risk characteristics suggested a moderate to high potential of HPV positivity among the women in the sub-district. This suggested high potential was confirmed by the high prevalence of HPV infections detected among these women as shown in table 4.14.

The finding that only one woman reported living with HIV, was far below expectations, since the national HIV sentinel site closest to the sub-district (Agormanya) had reported the highest HIV prevalence (7.8%) in Ghana between the year 2003 and 2011 (within the period this study was designed). Although some participants may not have reported their correct HIV status, this non-report was not likely the only reason for the very low reported HIV status in this study. The fact that the sentinel site closer to the sub-district reported high HIV prevalence may not necessarily mean that every community within its catchment area will have a high HIV prevalence.

5.1.3 Distribution of Reproductive Risk Characteristics

The assessment of the persistence of high risk HPV infections and the exposure to the reproductive risk characteristics are the most useful indicators of the potential of developing cervical lesion (Vaccarella *et al.*, 2006; Bosch *et al.*, 2008; Koshiol *et al.*, 2008). However, in the absence of data on HPV persistence, determining the proportion of the women at risk of developing pre-cancer lesions based on the distribution of the categories of the reproductive characteristics is considered useful in this study (International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Vaccarella *et al.*, 2006). To this end the sub-district was most likely a low-at-risk community for cervical lesion development. However, although the study was not powered enough for the estimation of the prevalence of cervical lesions, the non-detection of cervical lesions among the participants of this study, may be considered a confirmation of this most likely low-at-risk status (a further discussion on the non-detection of cervical lesion is presented subsequently) (Table 4.20). This deduction is supported by the distribution shown in table 4.3 and the discussions in section 2.5.2.2. Specifically, a history of oral contraception (OC) usage for 5 or more years is a high risk factor for the development and

progression of cervical lesions (Chelimo *et al.*, 2013; Luhn *et al.*, 2013; Almonte *et al.*, 2011; The International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Castellsagué and Muñoz, 2003b; Smith *et al.*, 2003) and in this study, it was determined that 23.3% of the women had ever used OC in the past 10 years, and only 7.7% had been using it for 5 or more years. These imply that only a small proportion of the women were at risk of developing cervical lesions and therefore, suggested that the sub-district was a community at low risk for cervical lesion. Furthermore, and as indicated in section 2.5.2.1, multiparity of more than 3 is a high risk characteristic for cervical lesion positivity (Chelimo *et al.*, 2013; Luhn *et al.*, 2013; Almonte *et al.*, 2011; The International Collaboration of Epidemiological Studies of Cervical Cancer, 2007; Castellsagué and Muñoz, 2003b), therefore, the finding of this study, which showed that 38.3% of the women had been pregnant for 5 or more times suggested that a small proportion of the women were likely to develop cervical lesion. Overall, the distribution of the sub-categories of reproductive characteristics were all suggesting that it was likely to detect cervical lesion among a small proportion of the women. However, the distribution of these categories of the reproductive risk characteristics, as indicated in sections 2.5.2 and as discussed for the sexual risk characteristics, was used as an indicator of the potential for cervical lesion positivity among the women, since the study was not powered to detect and quantify an association between the risk factors and cervical lesion positivity.

The other reproductive risk factors as shown in table 4.3; ever had an induced abortion, number of induced abortions, ever had a miscarriage and the number of miscarriage, have been shown not to be significant risk factors for high grade cervical lesion or cancer in some studies (Deacon *et al.*, 2000) but significant in others (Bahmanyar *et al.*, 2012). However, the observed

distribution of these risk factors revealed that more than half of the participants had not experienced both induced abortions and miscarriage (51.3% and 69.0% respectively) and among those who had experienced them, most did, only once (58.4% for induced abortion and 62.6% for miscarriage). Therefore, the community herein studied was suggested to be having most of the women in a low to moderate risk category for cervical lesions.

In summary and in respect of the first objective of this study, a moderate to high proportion of the women were potentially at risk for HPV positivity, but a small proportion of them were potentially at risk for cervical lesion positivity. This seemingly contradictory deductions are explainable by the following facts; that the sample size for this study was not calculated with an expected prevalence of cervical lesion but rather with an expected HPV prevalence (that is, the study was powered only for the detection of HPV prevalence). This implies that potentially the distribution of factors associated with cervical lesions in this study may have been lower than what it may have been, since the sample size was smaller than it would have been if the study had been powered for the detection of cervical lesion. Additionally, it must be noted that it is persistent HPV infection that leads to the development of cervical lesion, not just HPV infection. The persistence of the HPV infections was not estimable with these risk factors. Furthermore, the possibility of lower detection related to cytology testing in Ghana, as indicated by a study in Ghana (Handlogten *et al.*, 2014) may have contribute to the low detection in this study.

5.1.2 Participant Characteristics that may have influenced Condom Use

As indicated in section 2.5.1.4, the use of condom is a very important primary prevention method for STIs in general (WHO; Guideline Development Group, 2014; WHO; Chronic Diseases and Health Promotion Group, 2006) and for the prevention of HPV infection in particular, it is the

consistent use of condoms that is important (WHO; Guideline Development Group, 2014; WHO; Chronic Diseases and Health Promotion Group, 2006; Shields *et al.*, 2004; Shepherd *et al.*, 2000). Therefore, it is important to determine the demographic characteristics of the participants that may have influenced the non-use of condom for the purposes of tailoring education on condom use. It can be seen from table 4.7 that to a large extent the expected distribution and association (Domfeh *et al.*, 2008) with the use of condom were observed. That is, more young women (less than 29 years) than expected used condom, less women older than 30 years and less married women than expected used condom. Additionally, table 4.8 shows that fewer women than expected with sexual ages higher than 19 years (corresponding to women who were 38 years and older) and those with one (1) current male sexual partner used condoms. These are understandable, in that most women 30 years and older were likely to be married and/or starting a family and most married women are less likely to use condom, except for the few whose husbands (in this male dominated society) may agree to the use of condom as a contraceptive. In spite of these expected findings, the finding that the use of condom was not consistent was very important to cervical cancer prevention, since it is the consistent use of condom that reduces the risk of acquiring an HPV infection. Therefore, there is the need for further studies to assess whether the inconsistent use of condom was fuelled by limited knowledge on condom use, cultural and/or religious limitations, so that more education and other appropriate cultural and religion sensitive intervention may be implemented as part of a cervical cancer screening programme in the Akuse sub-district and potentially in Ghana. This will encourage the consistent use of condom, particularly among men, since in this society, the use of the male condom predominates and women often may not have the final decision on its use.

5.2 RESPONSE TO THE REPORTING STRATEGIES

5.2.1 Response Rates

As discussed in section 4.2.1, the 38 peer-recruited women were excluded from the related analyses, since they did not fit in any of the three reporting strategies used and would have biased the estimated response rate. Therefore, the findings show that the overall response rate attained in this study (60.4%), was determined with the 377 study recruited participants, and that this rate was relatively high. Considering each of the three reporting strategies of this study, which were, long appointment time (duration) to report to hospital, short appointment time (duration) to report to hospital and report at a location within a community), the response rate for the strategy requiring the women to report to a location within the community for specimen collection (strategy 2), which was 95.1% (Figure 4.1), implied a very high potential for being an effective strategy for improving response to cervical cancer screening activities. However, it is worth noting that by the nature of the implementation of this community reporting strategies, the participants had a short duration within which to report for specimen collection after completing the study questionnaire. Among other reasons, this probably made it easier for that large proportion of the participants to follow through to the end of the screening. The other reasons include the fact that the participants were allowed to either report in the morning (on their way to their regular daily activity) or later in the day (up to 7:00 pm) when returning from their daily activities, without it interfering with their regular activities.

On the other hand, the response rates obtained for the strategy 1, short duration and long duration, which were 46.6% and 38.5%, respectively, indicated that requesting the participants to

report to a hospital within a short or long duration of time (after a home based recruitment process and without providing incentives for the participants), holds a low potential for recording high cervical screening coverage in a hospital setting. This is because these response rates were lower than the lower limit of the global range of response rate for cervical screening, which have been reported to be between 48% - 88% (Rossi *et al.*, 2011; Virtanen *et al.*, 2011; Gok *et al.*, 2010; Sanner *et al.*, 2009). These response rates obtained with the strategy 1 suggest that such a strategy might not be helpful as a cervical cancer screening strategy for Ghana. Therefore, for the planning of cervical cancer prevention and control by screening in Ghana, a strategy that will make it possible for women to access screening within their community (particularly at CHPS compounds) and at times that will not interfere with their day's activities, such as that used in this study, and that the appropriate training is given to the health workers at these CHPS compounds, should be given a strong consideration, since its response rate was higher than the upper limit of the global range.

Compared to the response rates reported by recent studies of cervical screening in different geographical regions, 48% - 88% (Virtanen *et al.*, 2011; Rossi *et al.*, 2011; Gok *et al.*, 2010; Sanner *et al.*, 2009), the overall response rate attained in this study was considered relatively moderate. This is considered a good response rate because although a similar response rate (60.0%) was observed in a similarly designed cervical cancer screening study in the USA, where a home-based recruitment was followed with specimen collection (Castle *et al.*, 2011b; Scarinci *et al.*, 2010b), the study in the USA provided financial incentive to achieve this high response rate, while the study reported in this thesis did not provide any incentive. Incentives were not provided in the study reported in this thesis, so as to mimic a real world situation in order to

assess the likelihood of a woman, living within these communities, to respond to a regular cervical cancer screening activity. Furthermore, although the study in the USA did not indicate the duration within which the participants had to report to the hospital after recruitment, the results of the study reported in this thesis showed that this duration was very important in achieving the high response rate (coverage). This deduction was based on the fact that a relatively poor response rate (38.5%) was recorded for the strategy that gave the women a longer duration (over a month) within which to report to the hospital after recruitment.

Furthermore, the determined response rates after calling to remind participant who had not reported, 10.3 and 44.4%, suggested that encouraging non-responders to participate in cervical screening activities by re-inviting them with a phone call may only contribute marginally to improving response rate or coverage. However, this may perform better than re-inviting non-responders by the sending of letters through the mail because of the less efficient postal system and low literacy rate in such communities in Ghana. On the other hand, a study by Rossi et al., (2011) suggested that re-inviting non-responders with the option to perform the specimen collection at home potentially may result in a greater response. This was also emphasized in a review of studies that employed self-specimen collection (Snijders *et al.*, 2013). However, since the study reported in this thesis was not specifically powered to determine the differences in these strategies and their respective reporting rates, the observed difference, although significant, are only suggestive of the potential influence these strategies may have on the coverage of community based cervical cancer screening activities.

5.2.2 Participant Characteristics that may have Influenced Reporting

In spite of the fact that the study was not powered to compare the response rates, it was necessary to determine if any of the participants' characteristics may have influenced their response to each of these strategies (Agurto *et al.*, 2005). This will be useful in planning a cervical cancer prevention and control programme for identifying the group of women to be targeted with appropriately tailored education to encourage their participation in cervical cancer screening. The association of the demographic characteristics with each of the reporting strategies indicated that reporting to a location within the community and reporting after a long duration to the hospital for specimen collection were not influenced by any of the demographic characteristics of the participants (all the chi-square *p values* were > 0.05 ; Tables 4.4 and 4.6). On the other hand, for the strategy of a short duration within which to report to the hospital, the results suggested a possible influence by age, where women between the ages of 20 to 39 years reported the most (chi-square *p values* < 0.05 ; Tables 4.5). This age related reporting may have resulted from the fact that most of the older women were engaged in activities, including those of their occupation, and could not make time to visit the hospital during the working hour of a day. This may imply that in developing a cervical cancer prevention and control programme for Ghana that will involve collection of specimen at the hospital, the planning should consider tailoring the reporting time to suit the social and economic activities the women may be engaged in, to encourage them to report for cervical cancer screening within a short time after recruitment.

5.3 ADDITIONAL FINDINGS - SPECIMEN COLLECTION

5.3.1 Performance and Preference for Specimen Collection

Although not a specific objective of this study, it is often useful to describe the performance and preferences for specimen collection, if it is possible; these form one of the very useful information for planning screening activity. The findings of this study that showed that a high proportion of the participants, 89.7% ($n = 226$), opted for both specimen collection methods (self-collection and health personnel-collection), was indicative of a high acceptance of both specimen collection methods among women in the Akuse sub-district. However, the 7 women who did not opt for self-specimen collection did so because one was blind and the others were afraid of hurting themselves while using the self-specimen collection brush. On the other hand, the 18 women who did not opt for the health-personnel specimen collection indicated that fear and difficulties with the use of the speculum were the major reasons for their choice. The proportion of the women who performed self-collection (96.4%) and health-personnel specimen collection (90.9%) in this study, were slightly higher than those reported for other studies and therefore, confirms the high level of acceptance of both methods by the women. For some these reported studies the following were noted; a cervical screening campaign in Cameroon reported 29.0% and 62.0% performed self-collection and performed health personnel collection respectively (Berner *et al.*, 2013); a study in the Mississippi Delta region of USA reported 80.5% and 40.5% of the women performed self-specimen and health personnel specimen collection respectively (Castle *et al.*, 2011b); a randomised control trial of Mexican women reported 98.0% and 87.0%, performed self-specimen and health personnel specimen collection respectively (Lazcano-Ponce *et al.*, 2011); a study in Uganda among women who did not have access to regular screening services reported 80.6% were willing to perform self-collection (Mitchell *et*

al., 2011). Some other studies, of women who were non-attendees of regular screening programmes, have reported slightly lower self-collection performance rates of 64.9% and 39.1% (Virtanen *et al.*, 2011; Gok *et al.*, 2010; Sanner *et al.*, 2009).

Despite these high performance rates for both methods in this study, the participants clearly indicated a higher future preference (50.6%) for health personnel specimen collection and a much lower future preference (20.3%) for self-specimen collection, after they had experienced both methods. This finding confirms reports of some studies in literature, which showed a similar order of preference, but there are other reports by studies that show the converse (Schmeink *et al.*, 2011). For example, in a study of women in an urban community, 68.0% preferred health personnel collection while 32.0% preferred self-collection, after they had experienced both methods (Anhang *et al.*, 2005).

It is worth noting that differences in study design (that whether a pre-performance or a post-performance preference was determined) may influence the distribution of preference of participants. While the study reported in this thesis and that by Anhang *et al.*, (2005) showed a higher post-performance preference for health personnel specimen collection (discussed above), a study of African-American women, who were made to indicate their preference before performing one of the two methods, reported a higher pre-performance preference (64.7%) for self-collection (Castle *et al.*, 2011b), suggesting that what the participant may expect before they perform the specimen collection might not be what they experience having performed the specimen collection.

5.3.2 Reasons for and characteristics that may have influenced Preferences

The participants of the study reported in this thesis who preferred health personnel-collection stated that the health personnel had performed the specimen collection satisfactorily because they were professionals and experienced. The participants also stated that they were not confident about whether they used the self-collection device correctly. These and most of the reasons stated by the participants of this study, for their preferences, have been reported by other participants who preferred health personnel specimen collection in other studies (Berner *et al.*, 2013; Scarinci *et al.*, 2013; Petignat and Vassilakos, 2012; Cuzick *et al.*, 2012; Schmeink *et al.*, 2011; Mitchell *et al.*, 2011). On the other hand, the participants who preferred self-specimen collection stated that the use of the speculum during the health-personnel specimen collection produced slight pain and/or induced fear, the self-specimen collection process was simple and easier to perform and that concerns about privacy formed the bases for their preference. These reasons were similar to those reported in some other studies by participants who preferred self-specimen collection (Scarinci *et al.*, 2013; Schmeink *et al.*, 2011; Rossi *et al.*, 2011). However, it was interesting to note that some of the participants were not confident in their performance of the self-specimen collection, although they stated that it was simple and easy for them to use. The inconsistency between performance and preference suggests the need for educating participants on the peculiarity and usefulness of self-specimen collection in order to improve its acceptability as part of cervical cancer screening activities. This education should be directed at boosting their confidence regarding their performance of the self-specimen collection process.

It was also determined that the preferences for the collection methods were not associated with and therefore were not influenced by the demographics of the participants (p values > 0.05). It

has been shown by other studies that often the demographic of the participants are not significantly associated with preference for specimen collection (Gok *et al.*, 2010). In one of such studies, only older age was associated with lesser likelihood to prefer self-collection (Mitchell *et al.*, 2011). In another study, only higher educational status and ethnicity were associated with a higher likelihood to prefer self-collection (Anhang *et al.*, 2005). However, the participants who had ever had an abortion and those who had ever experienced menstrual difficulties were significantly more likely to prefer health personnel-specimen collection ($p < 0.05$). This may be so because these women were more likely to either have had a speculum examination or a similar medical examination in relation to their reproductive health and they therefore were more comfortable with the speculum examination.

5.4 HPV BURDEN WITH SC AND HPC

5.4.1 Differences in HPV Prevalences of SC and HPC

In order to answer the objectives related to how different the HPV prevalences and genotype detection obtained with self-collected specimen (SC) was from those obtained with health-personnel collected specimen (HPC), this cross-sectional study of women aged between 15 years and 70 years assessed the distribution of HPV genotypes using the PGMY-09/11 PCR and a microsphere-based method for the detection and typing of 46 mucosal-associated human papillomavirus type (Zubach *et al.*, 2011). The findings of the study showed a clear and often significant difference between the HPV data obtained with these methods, such that SC HPV prevalences were all higher than those of HPC HPV prevalences (Table 4.13). The overall HPV detected with self-collected specimen, 43.1%, was almost twice that obtained with the cervical

swab specimen collected by the health personnel. Similarly, the LR-HPV prevalence obtained with self-collected specimen (23.3%) was more than twice that obtained with the health-personnel collected specimen (9.9%). However, although it is often the case that the high risk HPV prevalence obtained with HPC is not significantly different (very comparable) from those obtained with SC (Snijders *et al.*, 2013; Zhao *et al.*, 2012; Petignat and Vassilakos, 2012; Schmeink *et al.*, 2011; Ogilvie *et al.*, 2010; Petignat *et al.*, 2007), the prevalence of HR HPV obtained with the self-collected specimen in this study (27.2%; 95% CI 25.0 - 34.0) was relatively much and significantly higher than that obtained with the health-personnel collected specimen (16.6%; 95% CI 14.0 -24.0). This finding apart from confirming the widely drawn conclusion that self-collection is an appropriated alternative for health-personnel specimen collection for HPV testing, leaves us with the following questions; 1) Is it the case among the women in these communities that vaginal HR HPV infection is higher than cervical HR HPV infections? 2) Is it the case that the health-personnel specimen collection was not able to achieve the appropriate performance as compared to the self-collection? Nonetheless, within the limits of the performance of these collection methods in this study, significant and higher prevalence of HR HPV with the SC gives a better indication of the potential risk status of the women than HPC in respect of the burden of HR HPV. This is because although the SC HR HPV infection may be in the vagina, such infections may be easily transferred to the cervix when the vagina sheds the viruses as part of the viral life cycle (WHO; Guideline Development Group, 2014; Bosch *et al.*, 2008), since the external os is exposed to vaginal secretion (WHO; Chronic Diseases and Health Promotion Group, 2006; Sankaranarayanan *et al.*, 2003).

The spectrum of HPV detectable with the assay used for this assay, 46 of the 54 genital types (Schmitt *et al.*, 2013; Zubach *et al.*, 2011) included all the known high risk (HR), probable high risk (PHR) and low risk (LR) HPV types. This implies that the prevalence of HPV reported by this study included as many HPV types as possible and therefore gave a good estimate of the HPV burden in the Akuse sub-district. Most studies, particularly studies in Ghana, have reported HPV data based on detection methods that were/are limited in the number of HPV types they could detect (Attoh *et al.*, 2010; Domfeh *et al.*, 2008) and limited in the general detection of HPV. This is because, often the general HPV first round primers used in the nested-multiplex PCR do show the desired band on the agarose gel detection system, but amplifies the specific HPV genotype if present, during the second round of the nested PCR during which the specific primers are used (Awua *et al.*, 2016). Therefore, it is possible that some HPV genotypes that do not have their specific primers used in the second round will not be detected. At most, 37 HPV types are targeted with most of these methods (Schmitt *et al.*, 2013; Koidl *et al.*, 2008; Liu *et al.*, 2009). The wider spectrum of detectability in this study also ensures that the epidemiological, clinical and molecular information of the low risk types do not become limiting to future understanding of the role and related changes of all types of HPV in cervical cancers in Ghana.

Compared to the HPV prevalence determined for most of Ghana's neighbouring West African countries, in community based studies that used cervical swabs specimen collected by health personnel, the 23.3% HPV prevalence obtained with the cervical swab in this study was found to be lower. For instance, a community based cross-sectional screening study in Benin, reported an HPV prevalence of 33.2% (Piras *et al.*, 2011), while a larger community study in Burkina-Faso reported an HPV prevalence of 54.0% among the HIV negative women in the study (Didelot-

Rousseau *et al.*, 2006). In a community-based study in Nigeria, an HPV prevalence of 26.3% was reported (Thomas *et al.*, 2004) and in a case-control study in Abidjan, Côte d'Ivoire, HPV prevalence among the population control was 31.1% (Adjorlolo-Johnson *et al.*, 2010). However, the HPV prevalence reported for a rural Gambia community, 13.4% (Wall *et al.*, 2005) and in a hospital based study in Ghana, 10.7% (Domfeh *et al.*, 2008) were lower than that determined in this study. The HPV prevalence reported in this study was similar to that reported (21.5%) in a meta-analysis of HPV infection among women with normal cytology for the West African region, which used 8 studies conducted in Senegal, Nigeria, Guinea, Gambia and Cote d'Ivoire (Bruni *et al.*, 2010). Compared to HPV prevalence reported for some communities in countries outside Africa, such as China, the HPV prevalence determined in this study was higher than those (13.3% and 14.3%) reported by two community-based studies (Wu *et al.*, 2013; Ye *et al.*, 2010). Therefore, the results of this study adds to the existing data suggesting the possible existence of geographical differences in HPV prevalence among countries of the West African region, although differences in study design (selection of participants), age range of women (for one study), methods of HPV detection and genotyping, and the HIV status of the participant have been shown to also contribute marginally to differences in HPV prevalence (Martín *et al.*, 2011; Conesa-Zamora *et al.*, 2009).

A similar pattern of difference were reported in a study of South African adolescents (Adler *et al.*, 2012). However, this higher prevalence with self-collected specimen was explainable with the fact that self-collection samples were more likely to have included the vulva, vagina and the cervix during the sampling process and therefore more likely to detect more HPV infections than the health-personnel collection specimen, which involved only the cervix (Gravitt *et al.*, 2011).

Also, the large difference in the LR HPV prevalence was explainable by the fact that these HPV types were more associated with the vaginal epithelium and as such most studies have detected more LR HPV with SC specimen (Gravitt *et al.*, 2011; Moscicki *et al.*, 2010). Although HR HPVs have been associated with both cervical and vaginal epithelium and they have been detected with closer frequencies between SC and HPC specimen (Schmeink *et al.*, 2011), It has been shown that if the HPV prevalences were higher for HPC than SC, it meant that the SC were not well or were sub-optimally performed (Gage *et al.*, 2011; Gravitt *et al.*, 2011; Bhatla *et al.*, 2009; Petignat *et al.*, 2007). An optimal performance of SC should involve a full insertion of the cytobrush such that it reaches the cervix and a full rotation of the brush, 3-5 times while touching the cervix. Another contributor, although minor to this difference, which is the extent of exfoliation of the cervical epithelium into the vagina, has been reported in numerous studies and reviews, (Gage *et al.*, 2011; Gravitt *et al.*, 2011; Bhatla *et al.*, 2009; Petignat *et al.*, 2007).

5.4.2 Age-Specific HPV Prevalence

As part of determining the differences in HPV prevalences between SC and HPC, the age-specific HPV prevalence was very important to use. Therefore, for this study, this assessment was part of the study objectives. The findings of this study clearly shows that the SC age-specific HPV prevalences (i.e. overall, LR, and HR HPV) were higher than those with HPC and that a unique pattern of age-specific HPV prevalence by SC was obtained for the Akuse sub-district. Specifically, the distributions of the prevalence of the overall and HR HPV with age were bimodal for the determination with HPC specimen, but with an additional marginal peak (at middle-age) for the determination with the SC specimen (Figures 4.4 - 4.6). However, the distribution of LR HPV by age was bimodal for both SC and HPC specimen (Figures 4.4 - 4.6).

Of a higher importance is the generally higher HR HPV prevalence with SC and the additionally observed peak at mid age. These implied that the HPV prevalence as determined with the SC specimen compared favourably to that determined with HPC specimen and was a better indicator for the age groups with the greater burden of HPV infection. By extension, it may provide a good estimation of the age group at a higher risk of developing lower genital tract lesions in future, if considered as a specimen collection methods in an HPV testing based cervical cancer screening programme in Ghana.

As has been widely accepted, the high prevalence among younger women (15-29 years) is related to sexual behaviour, particular those associated with the initiation of sexual activities (Richter *et al.*, 2013; Clarke *et al.*, 2011; Gage *et al.*, 2011; Bruni *et al.*, 2010; Chan *et al.*, 2010; Onuki *et al.*, 2009). The smaller middle-age (40-44 years) peak of overall HPV prevalence by SC specimen has been shown in some cohort studies, with the suggestion that in addition to the persistence of previously acquired HPV infection, the acquisition of new HPV infection by these women accounts for the peak in prevalence (Baussano *et al.*, 2013; Gage *et al.*, 2011). The second high (major) HPV prevalence peak among women aged 55-59 years determined with both SC and HPC specimen has been shown only in selected geographical regions. For instance, a very similar age-specific overall and HR HPV prevalence distribution has been reported in a community based study in China, where an initial major peak was observed among women aged 20-24 years, small increase among women aged 34-39 years, a second major peak among women aged 50-54 years (Ye *et al.*, 2010). A study in Nigeria showed a first peak and a second high prevalence among women aged between 20-24 years and 55-64 years and a decline above 64 years (Clarke *et al.*, 2011; Gage *et al.*, 2011). Another study in Nigeria showed two peaks for

women 24-29 years and 55-64 years (Clarke *et al.*, 2011; Gage *et al.*, 2011). A logistic regression analysis of the overall, HR and LR HPV prevalence stratified by age for each of the risk factors was not possible with the data of this study because the number of participants in the subgrouping were too small for most of the cross-tabulations. As such the risk factors that may have been associated with the peak prevalence were not identified. With a logistic regression analysis in a larger Nigerian study, no risk factor was identified to have had any significant association with the high prevalence among the age group of 55-64 years (Clarke *et al.*, 2011).

Studies from other regions of the world have shown either a single peak or a bimodal trend with a high HPV prevalence among older women (50 years or older). While a large study in Hong Kong showed a bimodal trends with the first peaks at a relatively older age (26-30 years) and the second peak at a relatively younger age (46-50 years) (Chan *et al.*, 2010), a study in a South African community with a high HIV infection rates showed a peak prevalence among women less than 25 years and a gradual decline in HPV prevalence with age (Richter *et al.*, 2013). Studies in Denmark, Spain, Italy, Canada and the Netherlands have reported a single peak HPV prevalence (20-24 years) with a subsequent a decline with increase in age (Jiang *et al.*, 2013; Ogilvie *et al.*, 2013; Kjaer *et al.*, 2008; Franceschi *et al.*, 2006). A study of Japanese women showed a bimodal trend with a first major peak among women aged 15-20 years and a continuously raising prevalence in women older than 50 years (Onuki *et al.*, 2009). A multi-centre study in China showed a bimodal HR HPV prevalence for all six centres with the first peak observed among women < 25 years and a second peak mostly among women 40-44 years (Wu *et al.*, 2013).

A meta-analysis of the age-specific HPV prevalence for different global regions have shown similar trends, with a second high peaks among older women (50 years or older) for both Central America and West Africa (Bruni *et al.*, 2010). Although the high prevalence among the older women is still not clearly understood, some studies have suggested that the peak could have been due to hormonal changes associated with menopause that reactivated latent HPV infection, although these studies did not indicate how HPV could remain latent in the cervical tissue. Other studies have suggested that this could have been due to changes in sexual behaviour at different ages of both the women and their male sexual partners, while other studies have stated that this could be a population specific cohort effect. This high prevalence may also result from an increased HPV infection due to the reduced protection (local immunity) and increased dryness of the female reproductive organ as a result of menopausal changes. For instance, during or after menopause, there is a reduction in the secretions of the female reproductive organs which are known to protect against infections and provide lubrication that reduce the occurrence/prevent microabrasion and subsequent HPV infection (Richter *et al.*, 2013; Clarke *et al.*, 2011; Gage *et al.*, 2011; Bruni *et al.*, 2010; Moscicki *et al.*, 2006). The result of the study reported in this thesis confirms the existence of this trend but did not investigate the possible factors that may account for the trend.

With respect to the difference in the age-specific HPV prevalence determined with SC and HPC specimen, a consistent variation was observed for LR HPV prevalence but not for HR HPV prevalence. The variation in the HR HPV prevalence (Figure 4.5), reduced with age, such that beyond the age of 49 years the same prevalence were determined for both SC and HPC. This observation was not clearly understood as it was expected that the prevalence determined with

SC and HPC was to vary consistently across all age groups as seen for LR HPV prevalence (Figure 4.5). Would this imply that HR HPV infection within the study population was common in both vaginal and cervical epithelia for women between the ages of 15 years and 44 years, and then it becomes more restricted to the cervical epithelia in older women (45-54 years)? In other words, is there an increase in HR HPV cervical tissue specificity among older women? On the other hand, does the variation in the difference imply that while there was a consistent rate of infection of both the cervical and vaginal epithelia by LR HPV with increase in age, the infection of the vaginal epithelia by HR HPV reduces with increase in age? Also, this may just be a cohort effect or an occurrence by chance.

Since the HR HPV prevalence determined with SC specimens were higher than that of HPC, it is strongly encouraged that SC be used in determining the burden of HR HPV infections in Ghana,. However, in light of the fact that this is the first study on SC for determining the burden of HPV in Ghana, additional data will strengthen the evidence for the use of SC. Additionally, since most studies on HPV in Ghana and most West African countries report HPV prevalence determined with HPC, the HPV data obtained with the HPC will be useful for both inter and intra-county comparisons.

5.4.3 Level of Agreement between SC and HPC

In respect of the level of agreement between the data generated with the specimen collection methods, the extent of agreement between HPV prevalences with SC and HPC specimen were assessed with a kappa analysis. The findings indicated that the level of agreement between the two methods was low to moderate. Specifically, the highest observed level of agreement, was moderate and it was with the detection of HR HPV ($k = 0.402$), while a lower to moderate level

of agreement was observed for the detection of overall and LR HPV ($k = 0.321$ and 0.328 respectively). Also, a high level of concordance (77.9%) between the two methods was observed with the detection of HR HPV and a moderate level of concordance (67.3%) for the detection of the overall HPV (Table 4.13). The McNemar's test indicated that these observed differences in prevalences and extent of agreements (concordance and discordance) were significant.

Other studies have reported high levels of concordance ($> 75.0\%$) for the detection of overall HPV infection with either HPC recording a higher prevalence than SC (Bhatla *et al.*, 2009; Petignat *et al.*, 2007) or SC recording a higher prevalence than HPC (Zhao *et al.*, 2012). However, a review had shown that a wide range of levels of agreement ($k = 0.24 - 0.96$) have been reported between SC and HPC for both overall HPV and HR HPV detection (Schmeink *et al.*, 2011; Stewart *et al.*, 2007) and that the use of different devices for the collection of SC and HPC and populations had an influence on the levels of agreement. Therefore, for the Akuse Sub-district of the Lower Many Krobo district, SC specimens will be an appropriate alternative to HPC specimen for use in determining the burden of HPV infections.



5.6 ASSOCIATION OF SEXUAL CHARACTERISTICS WITH HPV POSITIVITY

There is the need to study the risk factors that may be determinants of HPV infection positivity among the population of women alongside the determination of HPV prevalence. Therefore, although this study was specifically powered to determine HPV prevalence, data were collected to assess the associations between the sexual risk characteristics and the HPV positivity. This study compared only the directionality (increased odds or reduced odds) of the associations of

the sexual risk characteristics with HPV positivity (overall, HR, LR, single and multiple HPV infection) obtained with SC specimen and those obtained with HPC specimen. This was to determine which of the two collection methods will conform to expectations with regards to the directionality of the association. These comparisons were based on the directionality reported by studies that determined the associations between sexual risk characteristics and HPV positivity and reviews of such studies (Thomas *et al.*, 2004; Johnson *et al.*, 2012; Bahmanyar *et al.*, 2012; Oakeshott *et al.*, 2012; Almonte *et al.*, 2008; Chelimo *et al.*, 2013). Generally, because more HPV infections were detected with SC than with HPC and that for a similar sample size, an increase in prevalence leads to an increase in the power of a study, the directionality of the associations of the sexual risk characteristics with HPV positivity for SC were mostly as expected, while those for HPC were less often as expected. Once again, SC was more informative as compared to HPC. For instance, an increase in age at first sexual intercourse significantly reduced the tendency of being HR HPV and single HPV infection positive with the SC specimen, while for HPC specimen, it showed an equal tendency of being HR and single HPV positive. Similar trends were obtained for sexual age, as indicated in tables 4.15 and 4.16.



5.7 HPV VARIANT ANALYSIS

In determining whether the variants of the detected HPV16, HPV 18 and HPV 45 were of the African lineage and how similar they may be to those detected in other African countries, this study obtained a partial nucleotide sequence (~ 375 bp) of the long control region (LCR) of the genome HPV genotypes. This region of the HPV genome has been shown to be very informative

and therefore very useful in the study of HPV variants (Al-Awadhi *et al.*, 2013; Mendoza *et al.*, 2013; Cornet *et al.*, 2012; Tornesello *et al.*, 2011).

All the isolates of HPV 16 detected in this study were of the Africa lineages, and the commonest lineage (80%) was the HPV16 African 2b (Afr2b). Only 1 isolate was identified as an HPV16 Africa 2a, while the other three isolates were identified as belonging to the HPV16 African 1 lineage. The nucleotide position used in this analysis (7460 -7840) was shown not to have had enough information to clearly distinguish HPV16 Afr1a and HPV16 Afr1b since the previously described HPV16 Afr1a variants (KF466576) and HPV16 Afr1b (KF466592) that were included in the alignment were clustered closely together in the phylogenetic tree (Figure 4.6). These two lineages are distinguished from each other with the substitution at nucleotide position 7438 (A to C), which is characteristic to Afr1b (Cornet *et al.*, 2012), but this position was not part of the sequence range obtained in this study.

Although not clearly understood, it was interesting to note that a repeated duplicate sequencing of a single DNA specimen produced two the isolates GH618 and GH618_2, which varied at two positions (Table 4.17). However, most of the variability identified exclusive to the isolates of this study were located within the genome positions 7801 to 7828, which contain the binding sites for the transcription factors, activator protein 1 (AP1) and Octamer-binding protein 1 (Mendoza *et al.*, 2013). These may have implication for transcription activities in pathogenic pathways. For the 3 women whose SC and HPC specimen were both positive for HPV16 and had good sequences for analysis (three pairs), two pairs of sequences matched exactly. Specifically, the isolates GH511 (obtained with SC specimen) and GH759 (obtained with HPC specimen) clustered together as HPV16 Afr2b lineage, while the isolates GH517 (obtained with SC

specimen) and GH765 (obtained with HPC specimen) clustered together as HPV16 Afr2b lineage. The isolate GH618 (obtained with SC specimen) and isolate GH866 (obtained with HPC specimen) did not have their sequences matching exactly but clustered together as HPV16 Afr1 lineage. These imply the SC was performed well by the participants and also that the Rover sampler was effective at reaching and sampling the cervix uteri.

Of the 12 HPV18 genotypes sequenced, the African lineage 1 (Afr1) or the B1 and B2 lineages according to the new nomenclature were the commonest. The African lineage 2 (Afr2) or the B3 and C lineages (according to the new nomenclature) were also common. Interestingly, only a few were of the European lineage (Eur) or the A1 and A3 lineage according to the new nomenclature (Figure 4.7). In all, 41.7% (n = 5) of the isolates identified in this study had already been described by other studies (Chen *et al.*, 2013, 2009). Most of the nucleotide variations identified exclusively in this study isolates were located within the genome positions 7769 to 7714 and 7795 to 7814, regions where the binding sites for the transcription factors NF1, YY1 and AP1 are located. These changes may have implication for transcription activities in pathogenic pathways (Mendoza *et al.*, 2013). For the women whose SC and HPC specimen were positive for HPV18, only one pair had good sequences for comparison, Isolate GH561 (obtained with a SC specimen) and isolate 809 (obtained from the HPC specimen of the same woman), matched exactly and clustered as African lineage 1.

All of the 9 HPV45 genotypes sequenced were determined to belong to the HPV45 lineage A1 (Africa lineage). For the women whose SC and HPC specimen were both positive for HPV45, only two pairs had good sequences and these pairs of sequences, isolate GH583 (obtained with

SC specimen) and GH831 (obtained with HPC specimen), and GH632 (obtained with SC specimen) and GH880 (obtained with HPC specimen) matched exactly.

The fact that the HPV18 and HPV16 isolate were very similar to variants isolated from specimens collected in Burkina Faso (a country that shares a border with Ghana) but those of HPV45 did not match any previously described isolated from the West African region, may be informative to the understanding of HPV genetic variability, transmission and distribution within the West African region. Full genome sequencing will throw more light on these issues.

5.8 CERVICAL LESIONS AND THE ASSOCIATION WITH RISK FACTORS

Another objective of this study was to determine the prevalence of cervical abnormalities by Papanicolaou (Pap) smear screening and evaluate its association with the demographic characteristics and risk factors. However, no cervical lesion was detected among the participants of this study and therefore the association of cervical lesions with the studied risk factors and demographic characteristics could not have been determined. This may not be surprising in a study with this small sample size, because in older studies in Ghana with sample sizes of about 1000 women very low prevalence of cervical lesions, 0.6% low grade lesion, 0.6% high grade lesions and 1.6% high grade lesions, were reported (Grace *et al.*, 2006; Nkyekyer, 2000). Also, a study in South Africa (a country with high prevalence of cervical cancer) that studied about 20,600 women reported a 2.42% low grade lesions, 1.8 high grade lesion and 0.47% diagnosed invasive cancers (Fonn *et al.*, 2002). In Cameroon, a study of over 2000 women identified 2.1% low-grade lesions and 1.8% high grade lesion (Marie *et al.*, 2013). These suggest that the study

reported in this thesis is not powered enough to give a good estimate of the prevalence of cervical lesion in this district but then the non-detection of any cervical lesion, leaves us with the question, is the community herein studied really a low at risk community for cervical lesions, instead of the expected high at risk status based on the distribution of sexual and reproductive characteristics, and the determined HPV and STI prevalences?" It may well mean that Ghana needs an active cytology-based screening campaign to determine the prevalence of cervical pre-cancer lesions. Additionally, the high HPV prevalence determined with SC and HPC but a non-detection of cervical lesions should be understood in the light of the fact that (as indicated in section 2.7) it takes some number of years of HPV persistence for cervical lesion to develop. Also, only a small proportion of HPV infection may persist in order to lead to cervical lesion (Goldstein *et al.*, 2009; Kahn, 2009; Schiffman and Wacholder, 2009; Flores *et al.*, 2006; Schiffman and Castle, 2005) and whether these high HPV infections have persisted long enough to lead to cervical lesions was not determined in this study. Additionally, cervical lesion may also regress after some time; therefore the non-detection does not imply it never developed.

5.9 LIMITATIONS OF STUDY

Being one of the very few initial community based studies involving HPV testing and the first to evaluate self-specimen collection in Ghana, the information generated in this study of women in the Akuse sub-district has provided valuable background information on what to include and expect in the implementation of a cervical cancer screening programme in Ghana. However, data generated in this study may not completely be free of biases.

Although measures were taken to provide very private interactions during the completion of the study questionnaire, there was still the potential for selective response bias with the response to sexual and reproductive related questions. This is because some of the women had to be notified that the number of children, number of induced abortion and number of miscarriage they reported did not tally with the number of total pregnancies they had reported. Also, this study did not assess the sexual behaviour characteristic of the participants' male sexual partners which has been shown to possibly contribute to the HPV prevalence among women.

In general, this study was powered to determine the overall and genotype specific HPV prevalence and therefore, the findings reporting stratifications of the association of HPV infections with sexual risk characteristics should be interpreted with this in mind, as the numbers in some subgroups were very small. Due to the fact that all the women in this study were determined not to have cervical lesions, the intended investigation of the association of HPV infection status as well as the risk characteristics with the presence of cervical lesions was not achieved in this study.

It must also be noted that the HPV variant analysis was performed with partial sequences of long control region (LCR) and since full genome analysis may be more desirable for variant analysis and HPV classification, the isolates' lineage and or sub-lineage similarities may only be slightly modified after a full genome analysis.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSIONS

This study of women aged 15-70 years was able to show that more than half of the women were categorized among the HPV high risk sub-categories of each of the sexual risk characteristics, suggesting the Akuse sub-district was a community at high risk for HPV infection. However, only a small proportion of the women were categorized among the high risk sub-categories of each of the reproductive risk characteristics,

Overall, the reporting of the participant was random among each of the reporting strategies used in this study; in other words, no participant characteristic influenced the reporting of the women for specimen collection. Therefore, based on the findings of this study, the Akuse sub-district can therefore be said to have a high overall HPV prevalence, 43.1% (95% CI 38.0% – 51.0%) with SC specimens and 23.3% (95% CI 19.0% – 31.0%) with HPC specimens. Specifically, the prevalence obtained with HPC was not greater than the hypothesized 26.3%. These high HPV prevalences were particularly among women aged 20-24 years and 55-59 years. Also, the sub-district can be said to have a moderate to high proportion of multiple HPV infection, with HPC specimens and with SC specimens respectively. Generally, the SC method potentially is an appropriate alternative to the HPC method for HPV testing as shown by the extent of the

concordance with HPC, age-specific prevalences and the directionality of the association of sexual risk characteristics with high risk (HR) HPV positivity.

The variants of the HPV16, 18 and 45 were predominantly of the African lineage and similar to those reported in some West African countries. No cervical lesion (prevalence of and associations of cervical lesions with HPV positivity and known risk factors) was detected and therefore, assessments or analyses of the associations of cervical lesion and both HPV positivity and participant's characteristics were not performed.

This study presents the first age-specific HPV prevalence data and the first HPV sequence data for Ghana.

6.2 RECOMMENDATION

6.2.1 Policy

It is recommended that the Lower Manya Krobo District Health Directorate and Akuse Government Hospital, should intensify education on sexual risk behaviours and include information about HPV and cervical cancer prevention in the sub-district's Health Education on Reproductive Health. Additionally, attention should be paid to the education on consistent use of condom and how it is important in cervical cancer prevention.

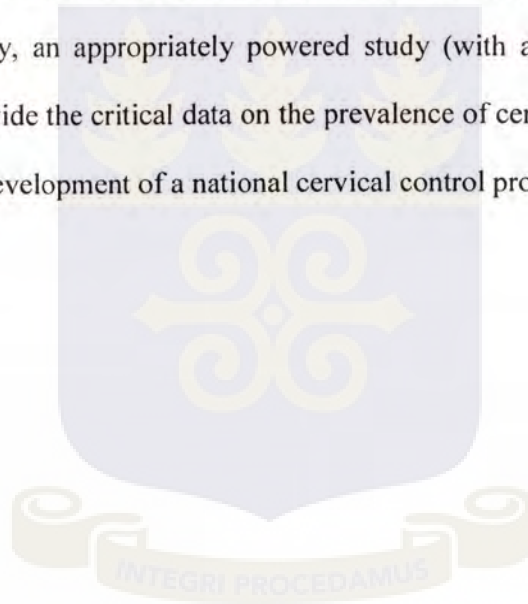
As has been shown in this study, the use of HIV prevalence (due to how it is determined in Ghana) will not be an efficient surrogate for determining communities at risk for HPV infection and cervical cancer. Therefore the identification of communities at risk by supporting studies such as the one reported in this thesis will potentially be useful to prioritizing cervical cancer screening programmes even if there be only limited resources. Furthermore, the inclusion of

HPV testing in such studies will be useful for the assessment of communities at risk of cervical cancer and therefore, useful for an effective screening programme. It is recommended that self-specimen collection (SC) and HPV testing are included in the development of a cervical cancer screening programme for Ghana. This is because SC provided a comparable HR HPV prevalence as that by HPC and with the potential of ensuring high attendance of women at screening, it will be key in the determination of communities at risk. Also, the inclusion of self-specimen collection will ensure that the epidemiological, clinical and molecular information of both high risk and low risk HPV genotypes do not become limiting to future understanding of the changing role of these genotypes in cancers and the efficacy of current HPV vaccines to be introduced in Ghana and those that might be introduced later. Furthermore, based on the moderate level of agreement and the high concordances of the HR HPV prevalence determined with self-collected and health personnel collected specimens in this study and the widely reported higher, need for training and retraining of personnel, the need for more health personnel and the need for attendance at a hospital, in relation to Pap testing, as well as the lack of time, discomfort, inconvenience, and cultural objections, a higher coverage in screening activity will be achieved in rural areas with self-collection while urban areas have Pap testing systems that will be referral sites for more detailed investigations. Also, the planning of messages for screening education should consider the major reasons and misconception regarding the women's preference identified in this study.

6.2.2 Further studies

In order to determine a very effective strategy for encouraging the participation of women in cervical cancer screening activities in Ghana and in order to determine whether different

communities will respond differently to these strategies, further investigation of the strategies identified in this study, particularly, the community based specimen collection strategy will help achieve effective screening in other communities. A larger study of the age specific HPV prevalence that will precisely determine various health characteristics such as immune markers, menopausal changes and sexual behaviour of male sexual partners will help throw more light on the observed high overall, HR and LR HPV prevalence among women aged 50 years or greater. A full genome analysis of the HPV variants identified with additional nucleotide variation in this study will help identify if those isolate are new lineages or new sub-lineages of their respective HPV genotypes. Additionally, an appropriately powered study (with a large enough samples size) is recommended to provide the critical data on the prevalence of cervical pre-cancer lesions to appropriately inform the development of a national cervical control programme.



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APPENDIX I

Table A.1: Primer sequence for round one genotyping PCR amplification

Primer designation	Primer sequence
PGMY11-A	GCA CAG GGA CAT AAC AAT GG
PGMY11-B	GCG CAG GGC CAC AAT AAT GG
PGMY11-C	GCA CAG GGA CAT AAT AAT GG
PGMY11-D	GCC CAG GGC CAC AAC AAT GG
PGMY11-E	GCT CAG GGT TTA AAC AAT GG
PGMY09-F	CGT CCC AAA GGA AAC TGA TC
PGMY09-G	CGA CCT AAA GGA AAC TGA TC
PGMY09-H	CGT CCA AAA GGA AAC TGA TC
PGMY09-Ia	G CCA AGG GGA AAC TGA TC
PGMY09-J	CGT CCC AAA GGA TAC TGA TC
PGMY09-K	CGT CCA AGG GGA TAC TGA TC
PGMY09-L	CGA CCT AAA GGG AAT TGA TC
PGMY09-M	CGA CCT AGT GGA AAT TGA TC
PGMY09-N	CGA CCA AGG GGA TAT TGA TC
PGMY09-Pa	G CCC AAC GGA AAC TGA TC
PGMY09-Q	CGA CCC AAG GGA AAC TGG TC
PGMY09-R	CGT CCT AAA GGA AAC TGG TC
HMB01b	GCG ACC CAA TGC AAA TTG GT

Table A.2: Geographic origin, lineage designation, length of complete genome and NCBI accession number of HPV types used for the alignment of the sequence obtained in this study.

Type	Isolate	Lineage	Sublineage	NCBI Accession #	Length	GC%
HPV16	HPV16Ref	E	(Prototype1)	NC001526	7906	0.365
	W0122b	E	(Prototype2)	AF536179	7904	0.368
	Qv02706b	E	Asian	HQ644261	7905	0.366
	BF325b	Af	Af-1	HQ644240	7908	0.363
	Rw918b	Af	Af-1	HQ644293	7908	0.363
	BF236b	Af	Af-2	HQ644239	7905	0.364
	Rw862b	Af	Af-2	HQ644292	7904	0.364
		Af	Af-2a	KF466626		
		Af	AFR1a	KF466576		
		Af	AFR1b	KF466592		
		Af	AFR2b	KF466652		
	Rw677b	Asian-American	AA1	HQ644289	7906	0.365
	Qv03545c	Asian-American	AA2	HQ644263	7906	0.366
HPV18	Ref	A	A1	AY262282	7857	40.44
	Qv29226	A	A1	KC470208	7857	40.41
	Qv32981	A	A2	KC470210	7857	40.38
	Qv26861	A	A3	KC470212	7857	40.32
	Z135	A	A4	KC470213	7857	40.35
	CU11	A	A5	GQ180787	7844	40.29
	Rw750	B	B1	KC470217	7824	40.04
	BF309	B	B2	KC470223	7824	40.07
	BF380	B	B3	KC470228	7844	40.01
	Qv39775	C	KC470229	7837	40.14	
HPV45	Ref	A	A1	X74479	7858	39.63
	Qv20214	A	A1	EF202156	7858	39.63
	Z79	A	A1	KC470250	7858	39.60
	BF208	A	A2	KC470255	7848	39.73
	BF134	A	A3	KC470256	7841	39.64
	Qv06560	B	B1	EF202163	7841	39.79
	Qv34163	B	B2	KC470260	7848	39.73

Isolate number pre_x denotes location from which sample was obtained, as follows. AS - Taiwan [1], BF - Burkina Faso [2], IN/INJP - Thailand [3], Qv- Costa Rica [4], R - USA (HAPI study) [5], Rw - Rwanda [6], W – USA (WIHS study) [7], Z - Zambia [8].



APPENDIX II

SCHOOL OF PUBLIC HEALTH, COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA

A Cross-Sectional Study

Title: Human Papillomavirus Infection and Cervical Lesions among Women in a High Risk Population; in 2 sub-district of the Lower Manya Krobo District, Ghana.

QUESTIONNAIRE A

For participant during household survey

1. Identification No. _____ Community : _____
2. House No. _____ Sampling house ID: _____.
3. Age: _____ Religion _____
- 4a. Marital status:
☐ Married ☐ Unmarried ☐ Cohabiting ☐ in a stable relationship
- 4b. If married, are you in a ☐ monogamous ☐ polygamous
5. Occupation _____
6. Educational status:
☐ No formal education ☐ primary ☐ junior secondary ☐ senior secondary
☐ tertiary
7. Age at first sexual intercourse _____
8. How many male sexual partners have you had since becoming sexually active? _____
9. How many male sexual partners do you currently have? _____
10. Number of pregnancies _____
- 11a. Have you ever had any abortion? ☐ Yes ☐ No
- 11b. If yes, please specify number _____
- 12a. Have you ever had any miscarriage? ☐ Yes ☐ No,

12b: If Yes, please specify number _____

13. Do you have menstrual difficulties? ☐ Yes ☐ No

14. Have you had any sexually transmitted infection within the past 10 years?

☐ Yes ☐ No

15. If yes, please select which, ☐ syphilis ☐ gonorrhoea ☐ candidacies
☐ HIV ☐ others

16. How often do you use condoms during sex?

☐ Every time ☐ Most often ☐ Intermittently ☐ Less frequently ☐ Not at all

17. Do you use oral contraceptives? ☐ Yes ☐ No

17b: If yes, how long have you been using it? _____

18. How often do you take alcohol?

☐ Every day ☐ only at occasions ☐ Not often ☐ Not at all

19. Do you smoke? ☐ Yes ☐ No

19b: if yes, how regularly do you smoke?

☐ Everyday ☐ Once a week ☐ Once a while

20. Have you ever heard of cervical cancer? ☐ Yes ☐ No

If yes, do know about the cause ☐ Yes, ☐ No

If Yes, please specify the cause _____

21. Do you know how it could be prevented ☐ Yes ☐ No

If yes please specify _____

22. Have you ever been tested by a Pap smear? ☐ Yes ☐ No

If yes, when _____ where _____

Thank you very much for participating in this study

QUESTIONNAIRE B

For Researcher at Sample Collection

A. Participant’s Identification No. _____ Community: _____

B. House No. _____ Sampling house ID: _____

Date for participant to see Doctor _____

24. Reported to Hospital ☐ Yes ☐ No Date _____

If No, why? _____

25. Been seen by Doctor for examination ☐ Yes ☐ No Date _____

If No, why? _____

26. Samples have been collected ☐ Yes ☐ No Date _____

If No, why? _____



QUESTIONNAIRE C

Self-Sampling for Participant after Sample Collection

A. Participant's Identification No. _____ Community: _____

B. House No. _____ Sampling house ID: _____

27. What is your opinion about the use of self-sampling devices?

- a. easy to use
- b. difficult to use

28. Which would you prefer?

- a. take your own samples
- b. health personnel to take your sample
- c. I have no preference

29. If you choose (a.) in 28 above, why?

- a. issues of privacy
- b. issues of culture belief
- c. issues of religious belief
- d. please specify _____

30. If you choose (b.) in 28 above, why?

- a. have no problem with gynaecologist's examination
 - b. self-sampling device is not user-friendly
 - c. I forgot how to use it
 - d. I was afraid I may hurt myself
 - e. I lost the device
 - f. other reasons please specify _____
-

APPENDIX III

CONSENT FORM

Title: Human Papillomavirus Infection and Cervical Lesions amongst Women in a High Risk Population in 2 District in the Eastern Region, Ghana

Principal Investigator: Adolf Kofi Awua

Address: Department of Epidemiology

School of Public Health

College of Health Sciences

University of Ghana

P.O. BOX LG13

Legon-Accra

Introduction

This Consent Form contains information about the research named above which is planned to run for three years. In order to be sure that you are informed about being in this research, we are asking you to read (or have it read to you) this Consent Form. You will also be asked to sign it (or make your mark in front of a witness). We will give you a copy of this form. This consent form might contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand.

Reason for the Research

You are being asked to take part in research to obtain data on the circulating or prevalence of Human Papillomavirus and cervical lesion in this district and evaluate the usefulness of a combination HPV testing and Pap smear testing for cervical cancer screening].

General Information about Research

Healthy women between the ages of 15 and 65 years would be asked to report to a health centre where cervical cell would be obtained from them using a cytobrush or cotton swab by routine method and a suspension in Phosphate Buffered Saline (PBS) prepared. These cell suspensions will be further analysed for HPV infection by genetic methods (PCR) and cytologic lesion by microscopy. Cervical swab samples will be stored for further research purposes in the areas of genotyping, HPV variant analysis and testing newer HPV detection methods when the need arises

Your Part in the Research

If you agree to be in the research, you will be interviewed and you will fill out a questionnaire that will seek for your demographic data, education background, sexual behaviour, reproductive health status and current knowledge of cervical cancer. You are to note that some of the questions are about your private and/or sex life and might be embarrassing and you may not have to answer any question if you do not want to. You will be asked to report to a specific health centre where cervical cell will be obtained from you by a trained health personnel and also, you would be directed as to how to obtain cervical cells by yourself both using cotton swabs or cytobrush by routine method. Your part in the research will last for two days. About (500 women) will take part in this research in two of districts of the Eastern Region of Ghana

Possible Risks

No physical, social and psychological risks are anticipated for participating in this study.)

[When applicable: These risks would be the same whether you got tested in this research or were tested without taking part in the research.]

Possible Benefits

Based on the results of the test, subjects who would need to go for further test and or treatment would be advised accordingly. The results of the research will be published so as to make it available to everybody to whom the information will be useful including national and district level policy makers.

If You Decide Not to Be in the Research

You are free to decide if you want to be in this research. Your decision will not affect the health care you would normally receive.

Confidentiality

We will protect information about you and your taking part in this research to the best of our ability. Your answered questionnaires and samples will be coded. These codes will only be known by the IP for the sake of finding you if needed be. You will not be named in any reports. However, the staff of the *School of Public Health, University of Ghana, Noguchi Memorial Institute for Medical Research and the Ghana Atomic Energy Commission* may sometimes look at your research records. Someone from the IRB might want to ask you questions about being in the research, but you do not have to answer them. A court of law could order medical records shown to other people, but that is unlikely.

[When applicable: If you miss a scheduled visit, we may contact you at home by phone, mail or in person to schedule another visit and to see if you still want to take part in the research. When this contact is made you will not be identified as being in this research.]

Compensation

You will not be paid, since you do not have to take part in this research.

Staying in the Research

When applicable: If you decide to take part in this research, we ask you to use only the research *(cotton swab or cytobrush that we provide*

Alternatives to Participation

You do not have to participate in the research in order to receive care or treatments.

Leaving the Research

You may leave the research at any time. If you choose to take part, you can change your mind at any time and withdraw

[When applicable] If so, please tell the research doctor/clinic staff why you wish to leave]

Also, you may be asked to leave the research if (list applicable points):

- the research doctor/clinic staff feels it is best for you, or
- you are not able to follow the research procedures, or
- the research is stopped.

[When applicable] we will tell you if we learn something new about (the research product or drug) that could affect your choice to stay in the research. When you are no longer in the research, you will still be able to use this clinic'

If You Have a Problem or Have Other Questions

Please call Professor R Adanu, (0244238556) if

- You get sick, or
- have concerns about your health (become infected), or
- have questions about the research

If you are sick or have a health problem due to your participation in this research, you will not have to pay for visits to see the research doctor/clinic staff.

VOLUNTEER AGREEMENT FORM

The above document describing the benefits, risks and procedures for the research title (Human Papillomavirus Infection and Cervical Lesions amongst Healthy Women Living within a High Risk Population in Ghana) has been read and explained to me. I have been given an opportunity to have any questions about the research answered to my satisfaction. I agree to participate as a volunteer.

Date

Signature of volunteer

Date

Thumbprint of volunteer

If volunteers cannot read the form themselves, a witness must sign here:

I was present while the benefits, risks and procedures were read to the volunteer. All questions were answered and the volunteer has agreed to take part in the research.

Date

Signature of witness

Date

Thumbprint of witness

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

Date

Signature of Person Who Obtained Consent



APPENDIX IV

SEQUENCE DATA

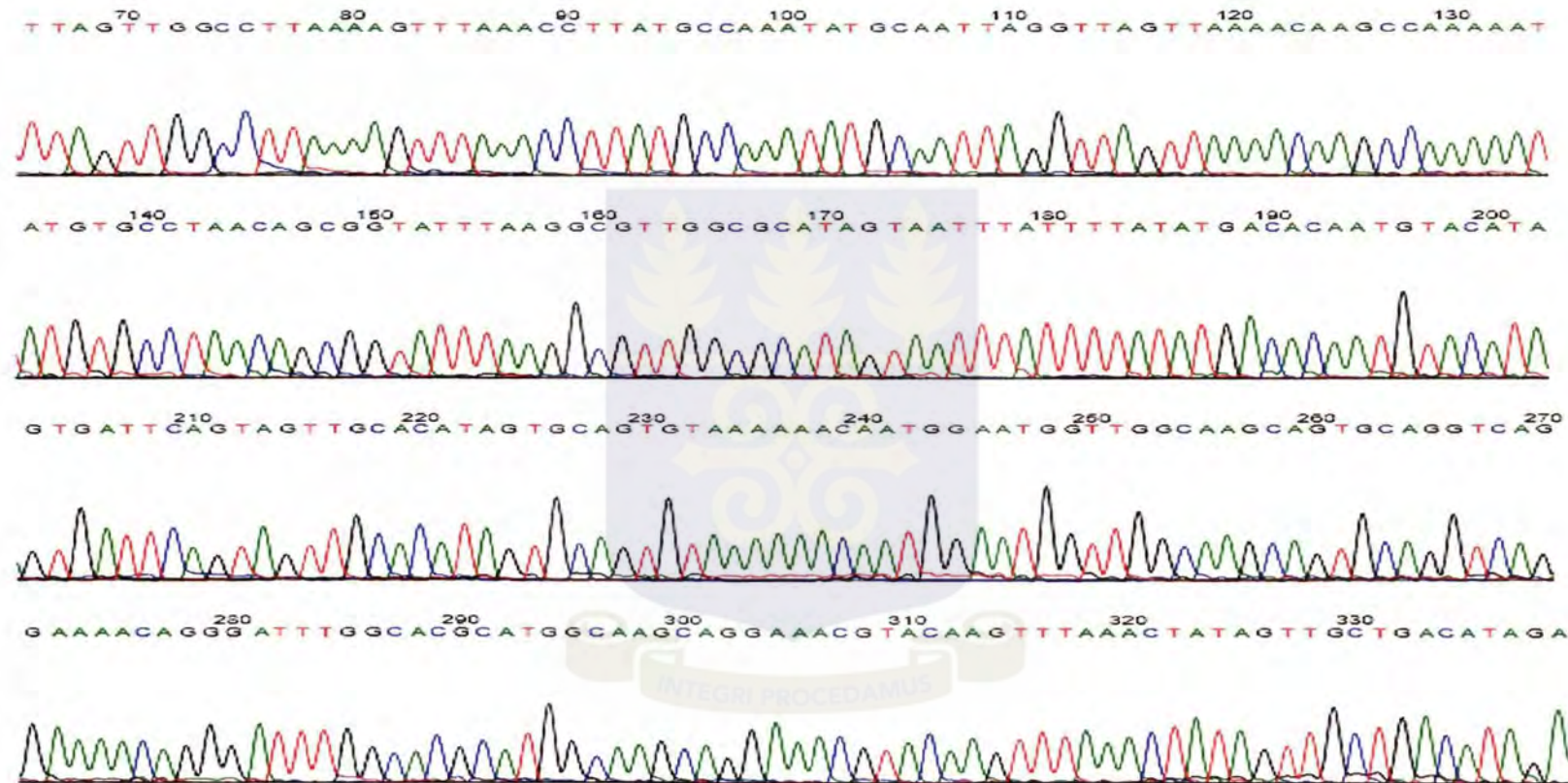


Figure A.1: Nucleotide sequence chromatogram (trace) of the partial LCR gene of the HPV16 Isolate GH609

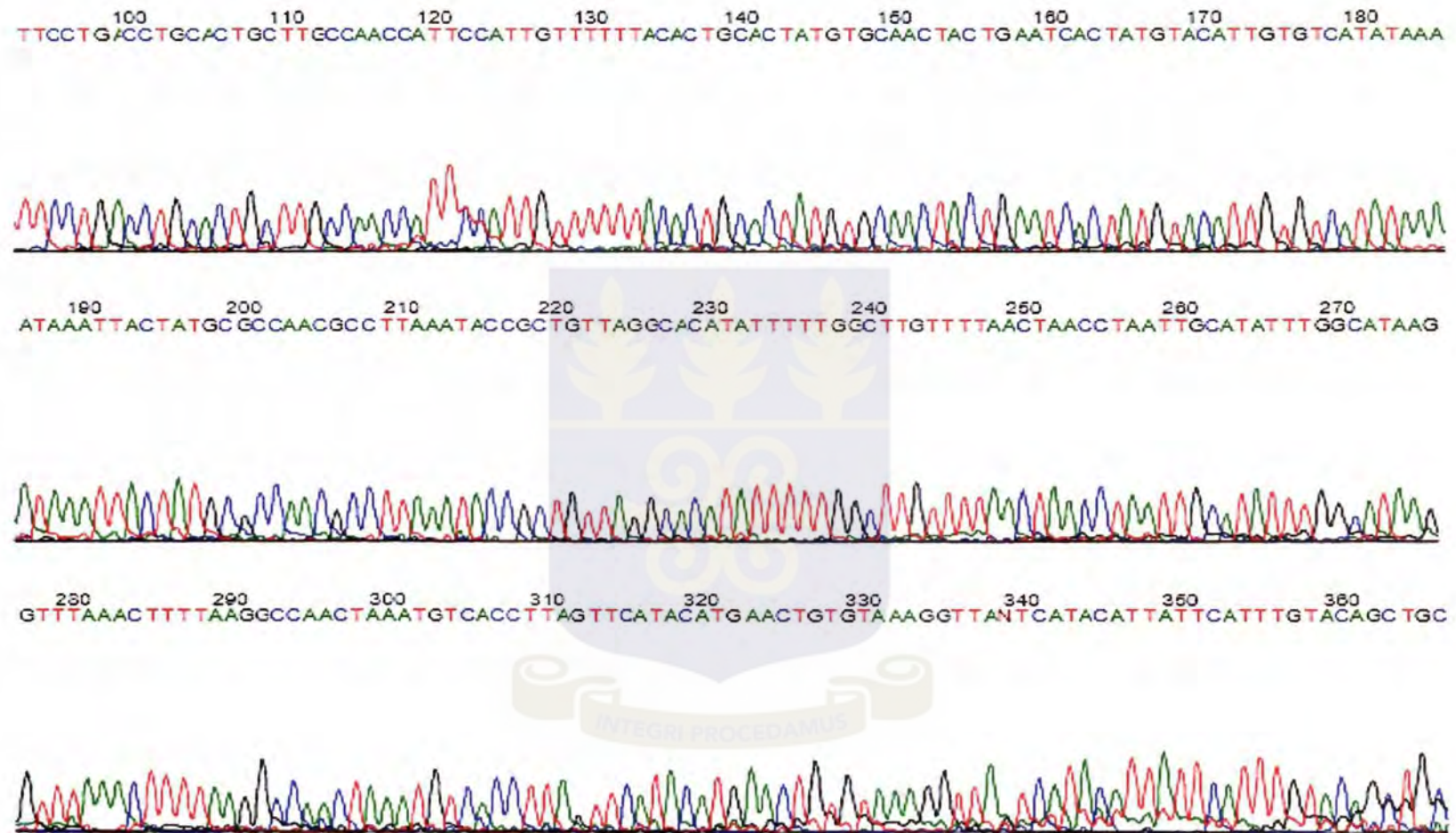


Figure A.2: Nucleotide sequence chromatogram (trace) of the partial LCR gene of the HPV16 Isolate GH517

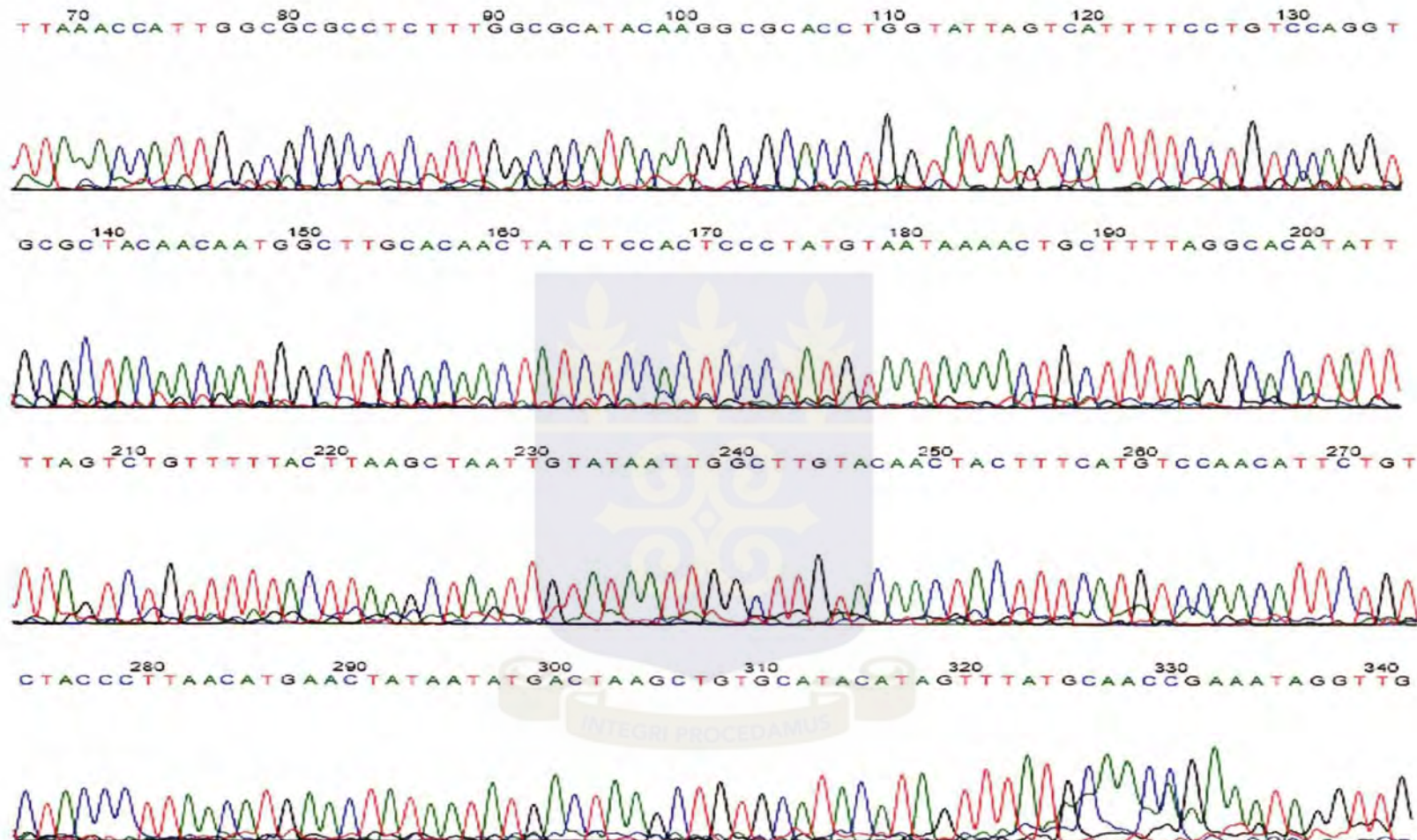


Figure A.3: Nucleotide sequence chromatogram (trace) of the partial LCR gene of the HPV18 Isolate GH561

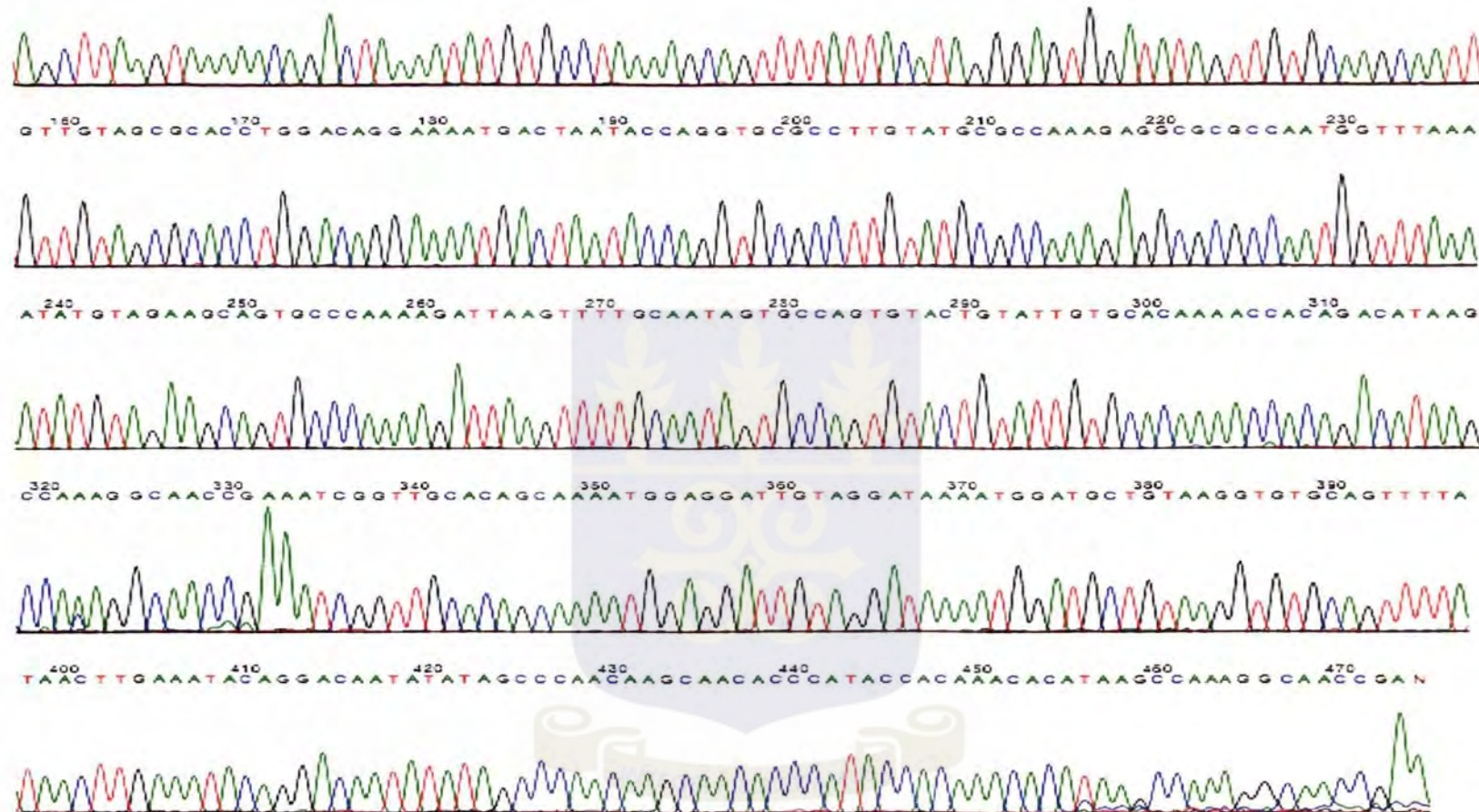


Figure A.4: Nucleotide sequence chromatogram (trace) of the partial LCR gene of the HPV18 Isolate GH627