SCHOOL OF PUBLIC HEALTH, COLLEGE OF HEALTH SCIENCES,

UNIVERSITY OF GHANA, LEGON

MOLECULAR EPIDEMIOLOGY OF HUMAN PAPILLOMAVIRUS INFECTION OF THE CERVIX IN WOMEN IN NORTH TONGU DISTRICT, VOLTA REGION, GHANA.

BY

GIFTY BOATENG

(10291585)

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DECLARATION

The work described in this thesis is an independent investigation conducted at the Department of Epidemiology and Disease Control, School of Public Health and University of Ghana under supervision.

All the work recorded in this thesis is original, unless otherwise acknowledged in the text or by reference cited. This work also has not in its present form or otherwise been submitted to this or any other university for the award of a higher degree.

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GIFTY BOATENG

(RESIDENT)

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PROF. E. K. WIREDU PROF. FRED BINKA

(SUPERVISOR) (SUPERVISOR)

DEDICATION

To my mother, Elizabeth Serwaa, my sister Dr. Mrs Evelyn Obeng Darko and all women living with cervical cancer

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Thanks be to God by whose wisdom and strength I have been able to complete this project. This thesis could not have been written without the generous help of my Supervisors, Prof. E. K. Wiredu and Prof. F. Binka.

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ABSTRACT

Background: Human papillomavirus (HPV) has been well established as the primary cause of cervical squamous intraepithelial lesion/cervical intraepithelial neoplasia and invasive cervical cancer. More than 100 genotypes of HPV have been identified and 40 types are sexually transmitted. Cervical cancer is the fourth most common cancer among women worldwide and about 85% of the cases occur in developing countries. In Ghana, cervical cancer ranks as the first most frequent cancer among women. Though cervical cancer is preventable, Ghana does not have an effective national cervical cancer screening programme for women to access. Prevalence and distribution of oncogenic HPV genotype varies greatly across inter and intra countries. In Ghana, few studies on the prevalence of HPV infection and pattern of risk factors have been conducted. There is also no knowledge of published data on the prevalence of HPV genotypes in general population. Therefore, this study sought to determine the prevalence and distribution of HPV genotypes in women in the North Tongu District.

Method: Five hundred women aged 15 years or older attending gynaecological clinic at Battor hospital and from three selected villages in the district were interviewed and cervical samples obtained after they gave their consent. Papanicolau smear test was performed and HPV DNA extraction, detection and genotyping performed using nested multiplex PCR technique. Prevalence of HPV and associations between exposures were determined.

Results: A total of 500 women with age range 15-70 years and a median age of 35 years were interviewed. The overall HPV prevalence among the women was 47.4% with 65% of these being infected with HR types only, 11.4% with LR types only and 23.6% with both HR/LR types. There was no age preference, HPV age-specific infection rate were young women (50%), middle aged women (49.6%) and old age (55%). Multiple HPV infection among the women was 51.9%. The five most common HR HPV types in either single or multiple infections were HPV-52 (13%), HPV-18 (11%), HPV-58 (8.6%), HPV-68 (7.2%) and HPV-51 (7.0%). HPV-43 (11.3) was the most common LR HPV type (58%). Cytology results were available for 453 (90.6%) women. Majority (98.9%) of the participants had normal Pap smears, with 0.4% presenting with high-grade squamous intraepithelial lesions, 0.2% ASCUS, 0.2% HPV infection and 0.2% squamous cell carcinoma. There was significant association between marital status and HPV positivity (χ^2=3.9, p=0.05) and in an unconditional logistic model, singles were significantly more likely to be infected (OR=1.9, 95% CI 1.03-3.32). There was also significant association between age at first sexual intercourse and HPV positivity ((χ2=8.9, p=0.03) and in an unconditional logistic model, women aged 14-17 years were 3 times more at risk of acquiring HPV infection (OR=2.8, 95% CI 1.40-5.58).

Conclusion: High HPV prevalence (47.3%) among women in North Tongu was detected. The five most frequent HR HPV types in the women were HPV-52, HPV-18, HPV-58, HPV-68 and HPV-51 in descending order. HPV-16 was detected in the only squamous cell carcinoma found. HPV-43 was the most frequent LR HPV and HPV-6/11 the least.

Multiple HPV infection was seen in about 52% of the HPV positive women. Marital status and age at first sexual intercourse were significantly associated with HPV infection. It is recommended that, Ghana Health Service should implement a national cervical cancer screening and prevention programme to reduce the incidence of cervical cancer in the country.

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LIST OF ABBREVIATIONS

AFENET-African Field Epidemiology Network

ACS-American Cancer Society

ACOG-American College of Obstetricians and Gynaecologists

ASCUS-Atypical Cells of Undetermined Significance

CDC-Centres for Disease Control and Prevention

CIN- Cervical Intraepithelial Neoplasia

DNA-Deoxyribonucleic Acid

dNTPs – Deoxyribonucleoside triphosphate

GAVI- Global Alliance for Vaccines and Immunizations

HR-High Risk

HSIL-High-grade Squamous Intraepithelial Lesions

HIV- Human Immunodeficiency Virus

HPV-Human Papillomavirus

IARC-International Agency for Research on Cancer

ICC- Invasive Cervical Cancer

ICO-International Cancer Organization

IUD-Intrauterine Device

KBTH- Korle-Bu Teaching Hospital

LSIL-Low-grade squamous intraepithelial lesions

LR-Low Risk

NMPCR-Nested Multiplex Polymerase Chain Reaction

OR-Odd Ratio

OC-Oral Contraceptives

PBS-Phosphate-Buffered Saline

PCR-Polymerase Chain Reaction

RR-Relative Risk

RNA-Ribonucleic acid

STIs-Sexually Transmitted Infections

SPSS-Statistical Package for Social Sciences

SCC-Squamous Cell Carcinoma

SIL-Squamous Intraepithelial Lesion

SD-Standard Deviation

UV-Ultra Violet

USPSTF-United States Preventive Services Task Force

URR-Upstream Regulatory Region

VLP-Virus like Particle

VIA-Visual Inspection with Acetic Acid

WHO-Wold Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Human papillomavirus (HPV) has been established as the primary cause of cervical squamous intraepithelial lesion (SIL)/cervical intraepithelial neoplasia (CIN) and invasive cervical cancer (zur Hausen, 2001). It has been implicated in 99.7% of cervical squamous cell carcinoma cases worldwide (Walboomers *et al*, 1999). Globally, HPV is one of the most common sexually transmitted infections (STIs) (de Sanjose et al, 2007). More than 90% of HPV infections are transient and cause either no detectable or mild pathological changes, but in some instances (5-10%) infections persist and can progress over the course of several years to SIL/CIN and then possibly to invasive cervical cancer (Brown and Weaver, 2013; Bosch *et al*., 2008 ; Ho *et al*., 1998). Some vulvar, vaginal, penis, oropharyngeal, and anal cancers are also manifestations of HPV infection (Kreimer *et al*, 2005; Parkin, 2006). Generally, HPV is thought to be responsible for about 85% of anal cancers, 70% of vaginal cancers, and 40% of vulvar and penile cancers (CDC Fact Sheets, 2008).

More than 100 genotypes of HPV have been identified and 40 types are sexually transmitted and infect the cervix and other anogenital regions (Herrero *et al*, 2005; de Villiers *et al*, 2004). Worldwide studies of HPV genotype distribution in various grades of cervical lesions and cervical cancer have identified 15 high-risk (HR) HPV genotypes: HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82 (Kreimer *et al*, 2005;

Parkin, 2006). Previous meta-analysis update showed that HPV 16 and 18 were responsible for more than 70% of cervical cancers worldwide, with the other most common genotypes being HPV-31, HPV-33, HPV-35, HPV-45, HPV-52, and HPV-58, showing varying regional prevalences (Mun˜ oz *et al*, 2003; Smith *et al*, 2007). Studies conducted by Clifford *et. al*, (2005) shows that, Sub-Saharan Africa has the highest prevalence of all HPV types and Europe the lowest. In all regions, HPV-16 was two times more common than any other high-risk type except sub-Saharan Africa, where HPV-35 was equally common. The second most common high-risk types in Asia were HPV-33 and HPV56 and in South America and Europe it was HPV58 and HPV31 respectively.

HPV is a small, non-enveloped virus, with a diameter of 55nm and belongs to the family *Papovaviridae*. It has an icosahedral capsid composed of 72 capsomers, which contain at least two capsid proteins, L1 (major) and L2 (minor). Each capsomer is a pentamer of the major capsid protein. Each virion capsid contains several copies (about 12 per virion) of the minor capsid protein. The HPV genome consists of a single molecule of double-stranded, circular DNA containing approximately 7900 base pairs associated with histones (Favre, 1975). The genome is functionally divided into three regions. The first is a noncoding upstream regulatory region (URR). This region contains the p97 core promoter along with enhancer and silencer sequences that regulate DNA replication by controlling the transcription of the "early" and "late" regions. The URR region also contains the highest degree of variation in the viral genome. The second is the "early"

region, which includes the genes E1, E2, E4, E5, E6, E7 and E8. This region is involved in viral replication and oncogenesis. Expression of the early gene products determines whether an HPV infection is active or latent, or leads to malignant transformation. The third is the "late" region, which encodes the L1 and L2 structural proteins for the viral capsid.

HPV transmission occurs primarily by skin-to-skin and skin-to-mucosa contact hence its association with sexual activity. The virus infects basal cells of stratified squamous epithelium with expression of various gene products intimately linked to epithelial cell differentiation. Other cells types appear to be relatively resistant. It is assumed that the HPV replication cycle begins with entry of the virus into the cells of the basal layer of the epithelium. Following infection of basal epithelial cells, the viral genome is maintained as a low-copy number episome by using the host DNA replication machinery to synthesize its DNA on average once per cell cycle (Flores & Lambert, 1997). The virus amplifies its DNA to high-copy number as the infected cell differentiates and migrates towards the epithelial surface where it synthesizes capsid proteins and causes viral assembly (Flores *et al*, 1999).

In high grade CIN and invasive cancers, HPV-DNA generally is integrated into the host genome. Integration of HPV-DNA disrupts or deletes the E2 region, which results in loss of its expression (Yoshinouchi *et al*, 1999). This normally reduces the regulation of the

transcription of the E6 and E7 genes leading to an increase in their expression. During a productive HPV infection, the function of the E6 and E7 products is to subvert the cell growth-regulatory pathways and modify the cellular environment in order to facilitate viral replication (Syrjänen & Syrjänen, 1999). The E6 and E7 gene products deregulate the host cell growth cycle by binding and inactivating two tumor suppressor proteins: the tumor suppressor protein (p53) and the retinoblastoma gene product (pRb) respectively. The inactivation of p53 and pRb proteins can give rise to an increased proliferation rate and genomic instability. As a result, the host cell accumulates more damage DNA that cannot be repaired, leading to transformed cancerous cells (Prak & Fujiwara, 1995).

The risk of contracting genital HPV infection and cervical cancer is influenced by a variety of factors and this has been shown in various epidemiologic studies (Richardson et al, 2003, Winer et al, 2003, Baseman *et al*, 2005). HPV infection is necessary but may not be sufficient for the development of cervical cancer. Cervical cancer therefore depends on a variety of additional factors that act in concert with cancer-associated HPV types. These factors include age, sexual activity, sexually transmitted infections, pregnancy, immunosupression and cigarette smoking (Vaccarella *et al*, 2006).

Age is an important determinant of risk of HPV infection. Most cervical cancers arise at the squamo-columnar junction between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix. There are continuous metaplastic changes at this site. The greatest risk of HPV infection coincides with greatest metaplastic activity

and this occurs at puberty and first pregnancy and declines after menopause (Burd E. M. 2003). The HPV prevalence reaches its peak in young adults (18-30 years) and declines at older ages (35-44 year) (Bosch et al, 2008). However, cervical cancer is more common in women older than 35 years, suggesting infection at a younger age and slow progression to cancer (WHO, 2010).

In addition to age, other studies have clearly indicated that the risk of contracting genital HPV infection and cervical cancer is influenced by sexual activity (Peyton et al, 2001). An individual is at greater risk of becoming infected with HPV if he or she had multiple sexual partners at any time or is the partner of someone who has had multiple sexual partners (Peyton et al, 2001). Sexual activity at an early age also has an increased risk of HPV infection, as does a history of other sexually transmitted diseases, genital warts, abnormal Pap smears in an individual or penile cancer in the sexual partner (Franco *et al*, 2001).

Cell-mediated immunity is the primary immune response to HPV infection; therefore, conditions that impair cell-mediated immunity such as renal transplantation or HIV infection increase the risk of acquisition and progression of HPV (Ho et al, 1994; Sun et al, 1997). In some studies, long-term use of oral contraceptives is also a significant-risk factor for high-grade cervical disease (WHO, 1993; Adam *et al*, 2000). Again, cervical cancer risk seems to be independently influenced by other variables including current

smoking and parity (Adam *et al*, 2000). Local immune suppression induced by smoking and the mutagenic activity of cigarette components have been demonstrated in cervical cells, and may contribute to persistence of HPV or to malignant transformation similar to that seen in the lung (Yang *et al*, 1996). Smoking appears to be the most important risk factor independent of HPV infection for higher grades of cervical disease (Adam *et al*, 2000).

1.2 Rationale of this Study

Cervical cancer and premalignant lesions constitute a major problem in women´s health. It is the fourth most common cancer among women worldwide (WHO, 2013), with an estimated 528,000 new cases and 266,000 deaths in 2012. About 83% of the cases occur in developing countries, representing 15% of female cancers. Cervical cancer is the most common female malignancy in sub-Saharan Africa (Parkin *et al*, 2003). In Western Africa, about 21.5% of women in the general population are estimated to harbour cervical HPV infection at a given time (WHO, 2010). The majority of cases are squamous cell carcinoma while adenocarcinomas are less common (Green *et al*., 2003).

In Ghana, cervical cancer ranks as the first most frequent cancer among women between 15 and 44 years of age (WHO, 2013). However, data is not yet available on the HPV burden in the general population. An estimated population of 6.57 million women greater than 15 years are at risk of developing cervical cancer (WHO, 2009). Current

estimates indicate that every year, 3052 women are diagnosed with cervical cancer and 1556 die from the disease in Ghana (WHO, 2013). Two new approaches for the prevention of cervical cancer have emerged over the past decade: vaccination for the primary prevention of HPV infection in adolescent girls and the use of screening methods to detect infection with carcinogenic HPV types, which allow for secondary prevention via the identification and treatment of precancerous cervical lesions and early-stage cervical cancers (Scarinci *et al*, 2010).

Cervical cancer prevention programmes have been cytology-based, but their success depends upon having more women covered in the right age group, implementing repeated quality controlled screening and developing excellent recall services for treating precancerous abnormalities (WHO, 2007). In low-resource countries, the capacity to implement this complex, high-resource protocol to cover entire populations has been a challenge. Alternative cost-effective screening and management options include visual inspection with acetic acid (VIA), with immediate cryotherapy of visible cervical lesions, and testing for HPV DNA (Goldie *et al*, 2005; Denny *et al*, 2005). These options also improve efficiency by limiting the steps a woman needs to take to access treatment (Denny, 2005). In several African countries including Ghana, early detection of cervical neoplasia by visual inspection with acetic acid was evaluated (Sankaranarayanan *et al*, 2001).

There are three prophylactic HPV vaccines that have been licensed globally; a quadrivalent and nanovalent vaccine marketed as Gardasil and a bivalent vaccine marketed as Cervarix. All are manufactured by recombinant DNA technology that produces non-infectious virus like particles (VLP) comprising of the HPV L1 protein, the major capsid protein of HPV. These have shown excellent efficacy against persistent HPV infection and related cervical lesions among women aged 16-24 years who have never been exposed to the virus (Villa LL *et al*. 2005). However, because of the heterogeneity of HPV genotypes in different parts of the world, the impact of the vaccines may vary across the regions. These vaccines are effective against oncogenic HPV types 16 and 18. In addition, the quadrivalent vaccines also targets HPV types 6 and 11, which cause low-grade cervical abnormalities and vast majority of genital warts (Villa LL *et al*. 2005) and the nanovalent target against HPV 31, 33, 45, 52 and 58.

In Ghana, a study conducted by Coleman et al., in 2011, shows that 40% of the participants had heard about HPV vaccine and 94% were willing to vaccinate their daughters or themselves (Coleman *et al* 2011). In 2013, a pilot project on Gardasil HPV vaccine was introduced in Ghana covering 17 districts in Central, Greater Accra and Northern regions. This is a two-year project supported by Global Alliance for Vaccines and Immunizations (GAVI) Alliance School girls aged 9-11 years were involved in the project (Osei 2013). Though cervical cancer is preventable, Ghana does not have an effective national cervical cancer screening programme for women to access.

Decreasing prevalence of the genotypes included in the HPV vaccine may affect the prevalence of other co-infecting genotypes due to the possible interplay between them in the cervical epithelium. As suggested by Woodman et al. in 2007, if different HPV genotypes compete to colonize the cervical epithelium, the prevalence of the genotypes not targeted by the vaccines could increase, changing the genotype-associated risks (Woodman *et al*, 2007). This suggests that the impact of the vaccine could also vary depending on the co-infecting genotype patterns.

Population-based studies of HPV genotype prevalence are therefore needed to predict how these approaches might influence cervical cancer prevention and how prophylactic HPV vaccination of young women could affect the secondary prevention of cervical cancer. However, there have been relatively limited or missing data from many regions in the world especially in the developing countries.

In Ghana, few studies on the prevalence of HPV infection and pattern of risk factors have been conducted in either gynaecological women or cervical cancer cases. Cervical HPV infection was reported in 33% and 48% of rural and urban women in Ghana, respectively using dot blot to detect the presence of HPV DNA (Szela *et al*, 1993). A survey at the gynaecology outpatient clinic of the Korle-Bu Teaching Hospital (KBTH), Accra, estimated the prevalence of cervical HPV infection in 75 women using PCR to be 10.7% (Domfeh et al, 2008). In 2014, Denny and his group assessed HPV prevalence and type

distribution in women with ICC. To address the lack of published data on the prevalence of HPV genotypes in the general population, this study sought to determine the prevalence and genotypes of HPV in women in the North Tongu district in the Volta region of Ghana which is important for current and future epidemiological studies. With the introduction of multivariate vaccines, there is the need to determine the baseline distribution of HPV genotypes and the current dominant genotypes in the Ghanaian population. In addition, the baseline distribution of HPV genotypes established in this study will be very helpful for monitoring potential HPV genotype evolution. Once vaccination has started, HPV typing remains necessary to monitor the changes in prevalence of the type(s) represented in the vaccines as a measure for vaccine efficacy.

The study will also provide data for the development of HPV screening test as a follow-up of abnormal Pap smear test in the country. This is because HrHPV testing is thought to improve cervical screening algorithms (Lorincz & Richart, 2003). HrHPV testing will also help in the management of women with cytologically equivocal smears (Cuzick *et al*, 2003; Bais *et al*, 2005), and the management of women treated for high grade CIN (Zielinski *et al*, 2004), although the treatment itself does not depend on the typing result.

Determination of country or community-specific risk factors for cervical cancer will allow for development, implementation, monitoring and evaluation of specific and targeted public health intervention measures to prevent HPV infection and thus cervical

cancer. Correlation of types cervical lesions with genotypes is expected to provide information on likely progression of disease caused by specific genotypes.

1.3 Overall Objective

This study sought to determine the prevalence of HPV genotypes in unscreened women in the North Tongu District.

1.4 Specific Objectives

1. To determine the prevalence and genotypes of HPV in HPV-unscreened women in North Tongu District.
2. To determine the HPV lesions using cytology in women in North Tongu District
3. To determine the association between cytology results and HPV infection rate.
4. To determine the extent of multiple HPV genotypes infection in women in North Tongu District.
5. To determine the prevailing risk factors associated with HPV infection in the district.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Human Papillomavirus

2.1.1 Structure of HPV

Human Papillomavirus (HPV) is a small, non-enveloped, double-stranded DNA virus with a diameter of 52-55nm (Williams *et al*., 1961). HPV virion contains an approximately 8000 base pairs circular genome enclosed in an icosahedral protein capsid composed of 72 capsomers and each capsomer is a pentamer of the major capsid protein (de Villiers *et al*., 2004). HPV is composed of open reading frames (ORFs); E1, E2, E4, E5, E6, E7, E8, L1 and L2 that are expressed at different times during its life cycle and so are categorized accordingly as early or late. These ORFs are functionally divided into three regions. The first is a non-coding regulatory region, termed as upstream regulatory region (URR) or long control region (LCR), which modulates viral DNA replication and gene transcription. The second is an early region (E1, E2, E4, E5, E6 and E7) which are involved in viral replication and oncogenesis. The third is a late region (L1 and L2), which encodes the major and minor capsid proteins. (Burd 2003, Stanley *et al*, 2007) Immortalization and transformation functions are associated with the E6 and E7 genes of high-risk HPV. E6 and E7 proteins from high-risk types are the primary oncoproteins; they manipulate cell cycle regulators, induce chromosomal abnormalities, and block apoptosis (Duensing & Munger, 2004).

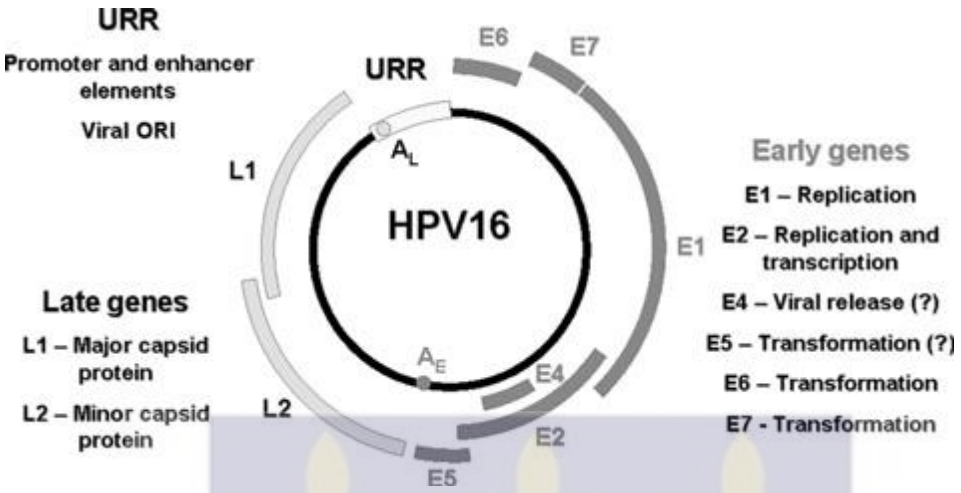


Figure 1: Genome Organisation of HPV16. Adapted from Stanley *et al*., 2007

2.1.2 Classification of HPV

HPV belongs to the Papillomaviridae family which is divided into genera, species, types, subtypes and variants (de Villiers *et al*, 2004; Favre, 1975). More than 100 different HPV genotypes have been isolated to date, and over 40 of these types infect the epithelial and mucosal lining of the anogenital tract and other areas (Schiffman & Castle, 2003, Bernard *et al*., 2010). HPVs are classified as "types" and numbers are assigned in order of their discovery (de Villiers *et a*l, 2004). Types are designated on the basis of the nucleotide sequence and homology of the L1 ORF. If the DNA sequences of the L1 genes differ more than 10% from the closest known HPV type, it is recognized as a new type. A subtype is defined with a 2-10% difference in the DNA sequences. Less than 2% is defined as an intra-type variant (de Villiers *et al*, 2004).

HPVs are contained within five evolutionary genera which include Alpha, Beta, Gamma, Mu and Nu (de Villiers *et al*., 2004). The two main HPV genera are the Alpha and Beta papillomaviruses, with about 90% of currently characterized HPVs belonging to one of these groups. Alpha papillomaviruses is the largest genera and it contains the genital/mucosal HPV types which includes 15 species. Genital HPV types are categorized into low-risk and high-risk types according to association wtith cervical cancer (Doordar, 2006). In a pooled data from 11 case-control studies of the association between cervical cancer and HPV infection from multiple countries, 15 HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82) were classified as high risk, three (26, 53, 66) as probable high-risk, 12 (6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108) as low risk and three (34, 57, 83) were considered to have undetermined risk (Munoz *et al*., 2003). HPV types 18, 39, 45, 59, 68 belong to species 7 whereas species 9 consist of HPV type 16, 31, 33, 35, 52 and 58 (de Villiers *et al*., 2004).

Beta papillomaviruses cause inapparent or latent cutaneous infections in the general population, but can become a problem in immuno-suppressed individuals and in individuals with an inherited defect such as epidermodysplasia verruciformis (EV). These patients may develop non-melanoma skin cancer (Harwood *et al*., 2002, Pfister, 2003)

2.2 HPV Infections

2.2.1 Life Cycle of HPV Infection

HPV initiates infection in the basal layer of the epithelium through minor abrasions in the skin and viral genome amplification occurs in keratinocyte differentiating cells using the cellular replication machinery (Doorbar, 2005). Once inside the host cell, the virus maintains its genome as a low copy number episome in the basal cell of the epithelium. The viral E1 and E2 proteins are expressed to maintain the viral DNA as an episome (Wilson et al., 2002) and to facilitate the correct segregation of genomes during cell division (You et al., 2004). There is increase in the proliferation of suprabasal epithelial cells once viral oncogenes, E6 and E7 are expressed. The E6 and E7 gene products deregulate the host cell growth cycle by binding and inactivating tumor suppressor proteins p53 and retinoblastoma gene product, pRB respectively (Syrjnen *et al*., 1999). When the keratinocyte reaches the superficial layer and dies, viral genomes are repackaged into capsids and shed from the cell (Doorbar, 2005; Stanley, 2006).

The time required for the keratinocyte to undergo complete differentiation and desquamation, that is, the time from virus infection to release has been estimated to be not less than three weeks. In humans, the time from infection to appearance of HPV induced lesions can vary from weeks to months (Doorbar, 2007). HPV infectious cycle effectively evades the immune system because there is no release of HPV antigens until the infected cell reaches the epithelial surface (Stanley, 2006; Wang, 2007).

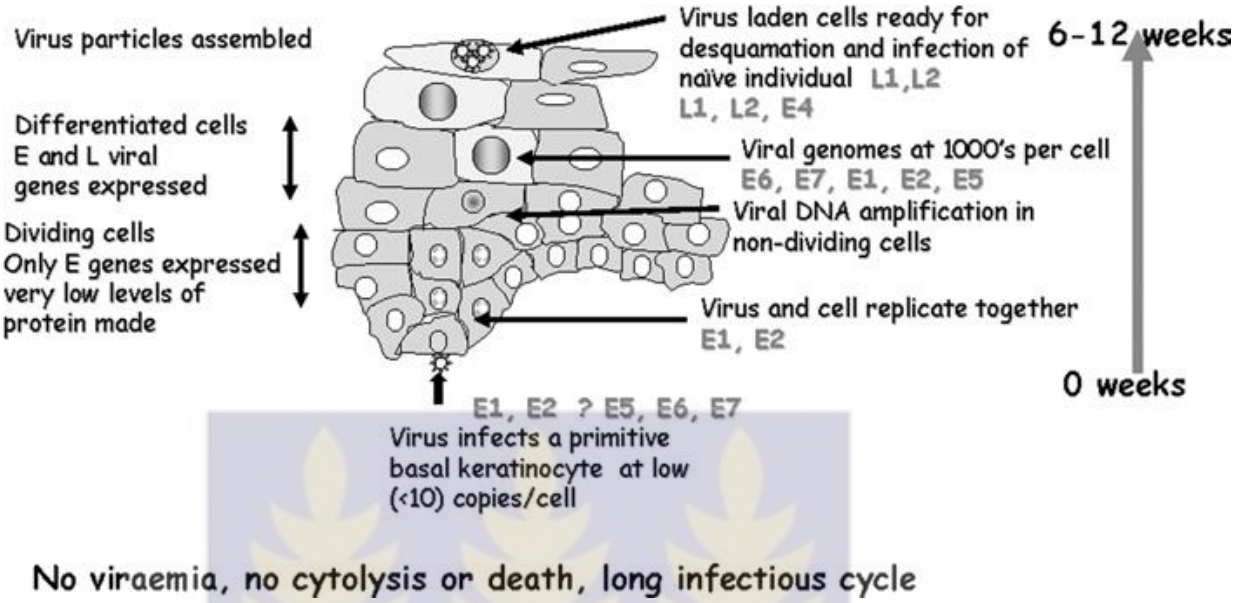


Figure 2: Life cycle of HPV. Adapted from Stanley et al., 2007

2.2.2 Manifestation of HPV Infection

Clinical infections of HPV are distinguished from subclinical and latent infections. Clinical HPV infections such as condylomata acuminata cause symptoms and are easily recognised. Clinical lesions are as a result of a productive HPV infection in the maturing epithelial cells. This leads to morphological changes in the infected epithelium, including cellular proliferation (epithelial acantosis) and degenerative changes in the nuclei and cytoplasm (koilocytosis) (Meisels, 1976; Purola and Savia, 1977).

Subclinical HPV infections are lesions which are visible under a colposcope and in histological specimens that demonstrate only minor epithelial changes that are not consistent with characteristic clinical HPV lesions (Syrjänen and Syrjänen, 2000). Subclinical HPV infections such as flat condylomata may be diagnosed by the presence of koilocytes or dyskeratocytes on cytology (Pap smear) which are specific morphological signs for permissive HPV infection (Meisels, 1983). Latent HPV infection is when the virus can only be detected by sensitive molecular methods such as PCR in an otherwise normal epithelium without any cytological, morphological or colposcopic alterations.

2.2.3 Transmission of HPV

Transmission of HPV occurs primarily by skin-to-skin or skin-to-mucosa contact. Several studies have shown that genital HPV is predominantly and largely transmitted through sexual intercourse (Kjaer *et al*, 2001). However, some clinical and epidemiological observations have shown that nonsexual transmission can not entirely be ruled out, but its implication in cervical cancer is likely to be marginal (Stevens-Simon *et al*., 2000). Sexual transmission is common for HPVs serotypes and because of that, they tend to be transmitted together (Plummer et al 2007) resulting in a high proportion (20–30%) of concurrent infections with several different types when women in the general population are sampled (Herrero *et al*, 2005).

The evidence for the non-sexual transmission of HPVs has been reviewed by several authors who have concluded thus: There is evidence of horizontal transmission of low-risk HPVs (Syrja¨nen & Puranen, 2000; Czegle´dy, 2001); Vertical and perinatal transmission of HPVs from mother to child also exist although, rates are small and vary widely (Duensing & Munger, 2004); Genital HPV infections, including genital warts, may occur in sexually naive populations such as virgins, infants, and children (Antonsson *et al*, 2003); High-risk genital HPV have been detected in non-genital mucosa, such as that in the mouth, oropharynx and conjunctiva (Rintala *et al*, 2005).

2.3 Detection of HPV Infection

HPV can be detected by several morphological methods, molecular methods and serological assays. However, HPV cannot be cultured. For any of these methods it is important to note the validity of the techniques, their sensitivity and specificity and the spectrum of HPV types detectable **(**Schneider 1993).

2.3.1 Morphological Methods

The following are the morphological methods:

- **Visual Inspection with Acetic Acid/Lugol's Iodine**

Visual Inspection with Acetic Acid (VIA) is a modification of a direct visual assessment of the cervix. VIA can be done with the naked eye (cervicoscopy), or with low-level magnification (gynoscopy). However, results from a South African study under the American College of Chest Physicians (ACCP) Portfolio, established that magnification did not improve the test performance over and above that of naked-eye visualization (Denny *et al*., 2002). VIA is based on histochemical reaction with better sensitivity than Pap smear, although its specificity is lower (Sankaranarayanan *et al*., 2005).

VIA involves inserting a vaginal speculum and swabbing or spraying the cervix with 3-5% acetic acid solution. Abnormal tissue such as that found in pre-cancerous or cancerous lesions will be affected by acetic acid and its color will change to white in the transformation zone (TZ), whereas normal cervix will appear unaffected. (McIntosh et. al., 2001; Sankaranarayanan & Wesley, 2001; Sellors et al., 2003). VIA test results are scored as VIA-negative, VIA-positive or suspicious for cancer (Sellors et al., 2002). VIA sensitivity and specificity range from 67–79% and 49-86%, respectively (Sankaranarayanan et al, 2005).

Visual Inspection with Lugol's Iodine (VILI) is similar to Schiller's iodine test which involves naked-eye examination of the cervix to identify mustard-yellow iodine non-

uptake areas after application of Lugol's iodine. The ranges of sensitivity and specificity for VILI among 10 cross-sectional studies conducted in Africa and India were 78-98% and 73-91% respectively (Sankaranarayanan *et al*, 2004).

- **Colposcopy**

Colposcopy is a descriptive diagnostic procedure developed by the German physician Hans Hinselmann in 1925 (Hinselmann H, 1954). The colposcope is a stereoscopic binocular instrument, which provides a magnified (4-40 times) 3-dimensional image Farley et al, 2005). The colposcope provides a magnified visual impression of the labia, vagina and the cervix and TZ. Although colposcopy was developed prior to widespread use of Pap smears as a screening method, it is now used as an addition to cervical cytology to enhance the diagnostic capabilities in women with an abnormal Papanicolaou test (Wright *et al*., 2002).

Pattern recognition in colposcopy is based on the evolution of surface contour, colour, degree of opacity, clarity of demarcation, and vascularity of the lesions. These criteria, interpreted according to a variety of metrics or grading systems, allow the provider to judge the clinical significance of the observed lesions (Farley *et al*., 2003). The original colposcopic terminology was reviewed and new terminology was adopted during Second World Congress of Cervical Pathology and Colposcopy in 1975. At the 11th World

Congress in June 2002, the International Federation for Cervical Pathology and Colposcopy approved a revised classification and terminology system that is currently in use (Walker *et al*, 2003).

- **Cervical Cytology**

Cervical cytology was introduced by George Papanicolaou into clinical practice in 1940 (Papanicolaou G.N. 1940). In 1945, Papanicolaou smear received the approval of the American cancer society as an effective method for the prevention of cervical cancer. There are two types of cytology; Conventional and Liquid-based. The commonly used one is the conventional.

- **Conventional Cytology**

Conventional cervical cytology is the most widely used cervical screening test. Cytology screening involves collection of cervical cell samples, followed by slide preparation, staining, reading, and reporting. Exfoliated cells from the vagina and uterine cervix are collected with a wooden spatula and a small brush (cytobrush), followed by fixation of the smear onto a glass slide and stained. Abnormalities in the Pap smear are classified using several classification systems with the Bethesda system (TBS 2001), currently the most widely used classification (Solomon *et al*., 2002).

On the accuracy of cervical cytology based on three reviews from 1995-2000, the mean sensitivity and specificity were 59% and 75%, respectively (Sankaranarayanan *et al*., 2005). In cross-sectional studies conducted in developing countries (Zimbabwe, South Africa and India) on test accuracy for cervical cytology, the sensitivity ranged from 44-78% and the specificity ranged from 91-96% (Sankaranarayanan *et al*., 2005).

- **Liquid-based Cytology (LBC)**

Liquid-based cytology (LBC) is a modification of Pap smear cytology, where the sample is collected from the cervix in the same way as with Pap smear cytology, but only plastic sampling devices may be used (Karnon *et al*., 2004). LBC relies on a fluid medium to preserve collected cervical cells. In this method the cervical sample is made into a suspension of the cells, which is then used to produce a thin layer of cells on the cytological slide.

The advantages of LBC include an increased possibility of a more representative and complete transfer of cervical cells from the sampling device to the slide and improved microscopic readability due to the elimination of problems such as poor fixation, air-drying artifact, uneven thickness of the cellular spread, debris from blood and inflammatory cells, and overlapping of cells. Cell suspension remaining after the preparation of the smear can also be used for additional testing procedures, such as HPV testing (Sankaranarayanan *et al*., 2005).

Studies shows that LBC improves sample adequacy and is probably more sensitive but less specific than Pap smear in detecting cervical neoplasia (Klinkamer *et al*., 2003). Although LBC has many advantages over conventional cytology, LBC has not been implemented in many low-resource settings (Sankaranarayanan *et al*., 2005).

2.3.2 HPV Deoxyribonucleic acid (DNA) Detection

Southern blot hybridisation was considered as the gold standard for reliable DNA identification of the various HPV types. However, between 5000 and 50000 HPV DNA copies should be present in a clinical sample for HPV detection. CareHPV is a rapid, highly sensitive DNA test that provides results more rapidly than other DNA tests. CareHPV detects 14 different types of HPV that cause cervical cancer. Another sensitive technique of HPV DNA amplification, the polymerase chain reaction (PCR) followed by simple methods of hybridisation was developed. This permits between 10 and 100 HPV DNA copies to be discovered (Van den Brule *et al*, 1992; Chan *et al*, 1992; Van Ranst *et al*, 1992). With PCR, HPV-type-specific primers enable the detection of one HPV type whereas consensus or general primers amplify a whole panel of different HPV types (Schiffman *et al*, 1991).

Since the adoption of PCR in 1985, many variations of the initial technique have been developed to improve the sensitivity, specificity, cost-effectiveness, and time consumption. One of the methods currently employed to save time and effort within the laboratory without compromising testing, is multiplex PCR. In multiplex PCR, more than

one locus is simultaneously amplified in the same reaction by adding more than one pair of primers. Multiplex PCR was first described in 1988 (Chamberlain *et al*, 1988) and since then it has been successfully applied in many areas of DNA testing, including analyses of deletions (Henegariu *et al* 1994) and quantitative assays (Mansfield *et al* 1993, Sotlar *et al*, 2004) .

Nested PCR is also a variant of PCR to increase sensitivity and specificity. This technique consists of the utilization of two pairs (instead of one pair) of PCR primers that are used to amplify a fragment. The first pair of PCR primers amplifies a fragment similar to a standard PCR. However, a second pair of primers called nested primers bind inside the first PCR product fragment to allow amplification of a second PCR product which is shorter than the first one. The advantage of nested PCR is that, if the wrong PCR fragment was amplified, the probability is quite low that the region would be amplified a second time by the second set of primers. Thus, Nested PCR is a very specific PCR amplification. In a study to develop a nested-polymerase chain reaction-restriction fragment length polymorphism (nested-PCR-RFLP) assay to detect and type HPV based on the analysis of L1 gene, of the total of 128 clinical samples submitted to simple PCR and nested-PCR for detection of HPV, 37 (28.9%) were positive for the virus by both methods and 25 samples were positive only by nested-PCR (67.5% increase in detection rate compared with single PCR) (Coser *et al*. 2011)

2.4 Natural History of HPV Infection

2.4.1 Clearance and Persistence

The majority of HPV infections are transient and asymptomatic and cause no clinical problems; 70% of new HPV infections clear within one year, and approximately 90% clear within two years (Molano *et al*, 2003). The median duration of new infections is 8 months (Molano et al, 2003). Persistent infection with high-risk types of HPV is the most important risk factor for cervical cancer precursors and invasive cervical cancer (Schlecht *et al*, 2003; Schiffman & Kjaer, 2003). The risk for persistence and progression to precancerous lesions varies by HPV type, with HPV 16 being more oncogenic than other high-risk HPV types (Moscicki *et al*, 2006; Wheeler *et al*, 2006). The time between initial HPV infection and development of cervical cancer is usually decades. Many aspects of the natural history of HPV are poorly understood, including the role and duration of naturally acquired immunity after HPV infection (Ault 2006).

2.5 Prevalence of HPV DNA

2.5.1 HPV Prevalence in Women with Normal Cytology

The global prevalence of HPV infection in women with normal cytology at any point in time is estimated to be 10.4% (de Sanjosé *et al*., 2007; Castellsagué *et al*., 2007); however, HPV prevalence has been found to vary with a range from 2% to 44% (Bosch and de Sanjose, 2003; Baseman and Koutsky, 2005; Herrero *et al*., 2005). The variability

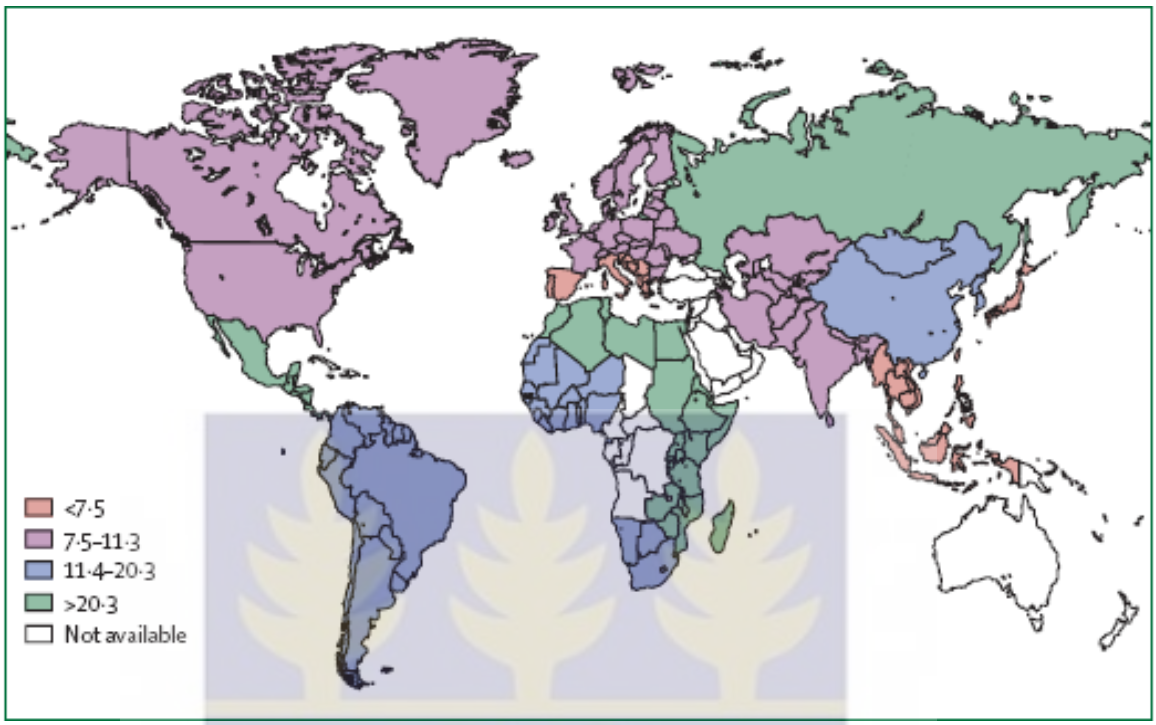
of results is considerable and relates to differences in the age range of the populations studied as well as on the sensitivity of DNA assays used for HPV detection. Two recent sources have provided estimates that may reflect the global prevalence, the age-specific prevalence and the type-specific prevalence as well as an approximation to describing the international variability (Clifford et. al, 2005, de Sanjose *et al*, 2007).

In a centrally coordinated international study, Clifford *et, al.*, (Clifford *et al*, 2005) used population-based sampling and centralised HPV-testing protocols from the International Agency for Research on Cancer (IARC) HPV prevalence surveys (Thomas *et al*, 2004) to compare HPV-type distribution in representative samples of women from 13 areas in 11 countries across sub-Saharan Africa, Asia, South America, and Europe. Sexually active women aged between 15 and 74 years, all with valid HPV test results and normal cytological findings were used in this study.

The overall HPV prevalence was lowest in Spain (1.4%) and highest in Nigeria (25.6%). After pooling by region, age standardised HPV prevalence was approximately five times higher in sub-Saharan Africa than in Europe, with intermediate prevalence in South America and Asia. The proportion of HPV-positive women with multiple infections ranged from 11·5% in Europe to 42·4% Asia. The most common HPV type, in either single or multiple infections, was HPV16, followed by HPV42, HPV58, HPV31, HPV18, HPV56, HPV81, HPV35, HPV33, and HPV45.

Sub-Saharan Africa had the highest prevalence of all HPV types and Europe the lowest. Although HPV16 prevalence was higher in sub-Saharan Africa than in Europe, HPV positive women in sub-Saharan Africa were less likely to be infected with HPV16 than were their counterparts in Europe, and were also more likely to be infected with other high-risk types and low-risk types. Risk of HPV18 infection among HPV-positive women was similar for all regions, although some heterogeneity for HPV18 was evident within the regions of South America and Europe. Heterogeneity was significant between regions for HPV31, HPV33, HPV35 (which was particularly prevalent in sub-Saharan Africa compared with other regions), HPV45, and HPV58. Heterogeneity was also evident within South America for HPV35 and within Asia for HPV58.

The second source is a comprehensive review of literature that estimated age and genotype-specific prevalence of cervical HPV DNA in women with normal cervical cytology worldwide by meta-analysis (de Sanjose *et al*, 2007). In this review, the global crude HPV prevalence estimate among women with normal cytology was 10·0%. The corresponding adjusted prevalence estimate was 10·4% (95% CI: 10·2–10·7). Africa, in particular eastern Africa, registered the highest adjusted HPV prevalence (31·6%, CI: 29·5–33·8) whereas Asia, in particular south eastern Asia recorded the lowest (6·2%, CI: 5·5–7·0). Figure 3 shows the estimates of the adjusted HPV prevalence for the different world regions, by quartiles of HPV prevalence.



Estimates are based on a meta-analysis of 78 studies including 157 879 women with normal cytology. Colours represent the adjusted prevalence in the region and denote the quartile distribution of all the estimates.

Figure 3: Estimated HPV DNA prevalence by World Regions (de Sanjosé *et al.*, 2007)

HPV prevalence estimates for 44 studies, stratified by region indicated that, HPV prevalence estimates were highest in women younger than 34 years and prevalence decreased in the 35-44 year-group for all major world regions (de Sanjosé *et al.*, 2007). However, there was an increase in the older age-groups (45–54 years and more than 54 years) in all regions, with the exception of Asia where rates continued to decrease.

Prevalence of HPV was higher in less developed countries (15·5%) than more developed countries (10·0%), and was highest in women younger than 25 years old (16·9%), decreasing with age thereafter. African women had the highest prevalence of HPV (22·1%, 20·9-23·4), and estimates were consistently high across all African studies.

The HPV type-specific prevalence of the five most common HPV types in women with normal cytology were HPV16 (2·5%), HPV18 (0·9%), HPV31 (0·7%), HPV58 (0·6%), and HPV52 (0·6%). HPV16 was the most common type in all regions with the exception of eastern Africa, and Japan and Taiwan where the most common type was HPV52. However, the degree to which HPV16 predominated over other types varied by region. HPV-18 is the second most frequent detected type after HPV-16 globally; however, in Africa, HPV-52 is the second most frequent type followed by and HPV-18. Among infected women, one-third of infections were caused by HPV16 or HPV18, or both. In Africa HPV types -58, 31, 35, 42, 66, 53, 45, 56 and 33 are frequently observed, while HPV-51, 71, 70 and 62 are frequently identified in other world regions. (de Sanjose *et al*, 2007).

2.5.2 HPV Prevalence in Women with High-grade Cervical Lesions and Invasive Cervical Cancer

In a meta-analysis study conducted by Clifford *et. al*., the estimated HPV prevalence was slightly higher in SCC cases (87.6%) than HSIL (84.2%). HPV16 was the most common type in both SCC (54.3%) and HSIL (45.0%) followed by HPV18 which was also more

prevalent in SCC (12.6%) than in HSIL (7.0%) (Clifford *et al*., 2003). In a similar study conducted by Smith et al., although HPV prevalence in both HSIL (87%) and ICC (85%) were similar, there was a 50% gain in information on HPV type distribution in ICC over the previous meta-analysis. This gain in information was particularly high for Africa and Asia, making overall estimates of HPV type distribution from these continents more robust and representative than before. About 70% of ICC cases were associated with either HPV16 (55%) or 18 (15%) infection. The six next most common types, namely HPV31, 33, 35, 45, 52 and 58 accounted for an additional 18% of cases. As a result of the accumulation of additional published data, differences by continent became less pronounced than those noted in the previous analyses with the estimates for the proportion of ICC cases attributable to HPV16/18 in Africa, Asia and South/Central America all increasing from the previously reported 59–64% (Smith *et al*., 2007). The eight most common HPV-types found in women with ICC are all included either in species 7 (HPV18, 45) or species 9 (HPV16, 31, 33, 35, 52, 58) (Bosch *et al*., 2008).

In Ghana, studies conducted by Attoh and his team (2010) showed that, of the 50 samples genotyped for HPV, 49 (98%) were determined as positive for HPV DNA. Eight HPV genotypes were detected and all were high-risk types. In decreasing order of prevalence, were HPV 18 in 42 (84%) samples, HPV 16 in 12 (24%) samples, HPV 45 in three (6%) samples, HPV 39 in two (4%) samples and HPV 35, 52,56 and 66 in one (2%) sample each. Multiple infections were detected in 13 (26%) of the 50 samples, the most common co-infections being HPV types 16/18 (18%). Triple infections were found in two (4%)

samples with HPV types 16, 18 and 39. HPV type 18 was more relatively frequent in adenocarcinomas and adenosquamous carcinomas than in squamous cell carcinomas (Attoh *et al*. 2010).

2.6 Co-infection with Multiple HPV Types

Many epidemiologic studies have indicated that co-infection with multiple HPV types is common. This has been observed more frequently among younger women (Molano *et al*, 2002; Castellsague *et al*, 2001) and among those with cytological abnormalities (Herrero *et al*, 2000). In the Brazilian Ludwig–McGill cohort, multiple types were detected at the same visit in one-fifth of all women who tested positive for HPV at any time during follow-up (Rousseau *et al*, 2001).

Moreover, it seems that infection with a given type does not decrease the probability of being infected by phylogenetically related types. In a study on concurrent and sequential acquisition of multiple HPV types in a population of young, female, American university students, it was found that acquisition of multiple infections occurred more often than expected by chance. Also, the risk of acquiring a specific HPV type was not substantially decreased among those with prior infection with a phylogenetically related type (HPV: 16 and 31; 18 and 45; 6 and 11) (Thomas *et al*, 2000).

In general, infections with single and multiple HPV types have comparable clearance rates. However, it is difficult to make cross-study comparisons because the definition of persistence varies appreciably across investigations. Perrons *et al*. (2005) found that infection with multiple types of HPV was associated with persistent HPV infection. Woodman *et al*. (2001) found that simultaneous infection with HPV 16 and another type resulted in longer duration of an HPV 16 episode as compared to single infection with HPV 16. However, not all data support this. Rousseau *et al*. (2000) observed that persistence of HPV infection was independent of co-infection with other HPV types. Liaw *et al*. (2001) found that the presence ofHPV16 was associated with an excess risk for acquisition of other types without affecting the persistence of the episodes with the additional types.

In some studies, infections with multiple HPV types have been associated with a higher risk of cervical intraepithelial neoplasia (Sasagawa *et al*, 2001; van der Graaf *et al*, 2002). However, in other studies, no increased risk of cervical intraepithelial neoplasia or cervical cancer was reported among women with multiple infections compared with women with single HPV infections (Bosch *et al*, 2002).

2.7 Association of HPV Infection and Cervical Cancer

2.7.1 Cervical Cancer

Worldwide, cervical cancer is the fourth most common cancer in women after breast cancer and the most common cancer in women in developing countries (IARC, WHO, 2013). In developed countries, cervical cancer accounts for 1.7% of all cancers while in developing countries it is 7% (Parkin, 2006). In 2012, the World Health Organisation estimated that there were 528,000 newly diagnosed cases of cervical cancer and this disease was associated with 266,000 deaths annually. Eighty percent of these deaths occur in developing countries (IARC, WHO, 2013). The higher prevalence of cervical cancer in developing countries may be largely attributable to the limited access women in these countries have to screening programs combined with high-risk characteristics such as poor nutrition and high parity (IARC, WHO, 2013). In the United States, prevalence and mortality rates vary according to ethnicity (Sataiya *et al*, 2007). There is a 1.5-fold higher incidence of cervical cancer and a 2-fold higher cervical cancer-related mortality rate in Black compared to Caucasian women (Bethesda, 2004). In Ghana, it is the leading cancer incidence and mortality (Bruni *et al.,* 2015; Farley *et al.,* 2015) (IARC, WHO, 2013).

Although HPV infection usually is asymptomatic, cervical infection can result in histologic changes that are classified as cervical intraepithelial neoplasias (CIN) grades I, II, and III or squamous intraepithelial lesions (low or high) on the basis of increasing degree of abnormality in the cervical epithelium or adenocarcinoma in situ (AIS).

Spontaneous clearance or progression to cancer in the absence of treatment varies for CIN 1 and CIN 2, and CIN 3. CIN 1 usually clears spontaneously (60% of cases) and rarely progresses to cancer (1%); a lower percentage of CIN 2 and 3 spontaneously clears (30%-40%), and a higher percentage progresses to cancer if not treated (>12%) (Ostor, 1993).

2.7.2 HPV and Cervical Cancer

The association between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold zur Hausen (zur Hausen, 2000). Since then, the association between high-risk HPV types and cervical squamous cell carcinoma has become well known. Worldwide, HPV has been implicated in 99.7% of cervical squamous cell carcinoma cases (Walboomers *et al*, 1999). The magnitude of this association is higher than that for the association between smoking and lung cancer (Franco, 1995). Adenocarcinomas of the cervix are also related to HPV (especially HPV-18), but the correlation is less pronounced and is age dependent. In women younger than 40 years, HPV was present in 89% of adenocarcinomas, whereas in women aged 60 years and older, HPV was observed in only 43% (Andersson *et al*, 2001).

The HPV types causing cervical cancer vary from one country to another. However, in any given country, over 70% are caused by two types, HPV16 and 18 (Munõz *et al*, 2003; Smith *et al*, 2007). In a pooled analysis of data from 11 case-control studies of women with histologically confirmed squamous cell cervical cancer conducted in nine different countries, HPV DNA was detected in 90.7% and 96.6% of the cases using MY09/MY11 and GP5+/6+ consensus primers respectively. The proportion of control

women testing positive were 13.4% and 15.6% respectively. Again in this study, the most common HPV types in patients, in decreasing order of frequency, were types 16, 18, 45, 31, 33, 52, 58, and 35. Among control women, types 16, 18, 45, 31, 6, 58, 35, and 33 were the most common (Munõz *et al*, 2003).

In determining HPV genotypes prevalent in Ghanaian women with cervical cancer, eight high-risk HPV genotypes in decreasing order were 18, 16, 45, 39, 35, 52, 56 and 66 (Attoh *et al*. 2010).

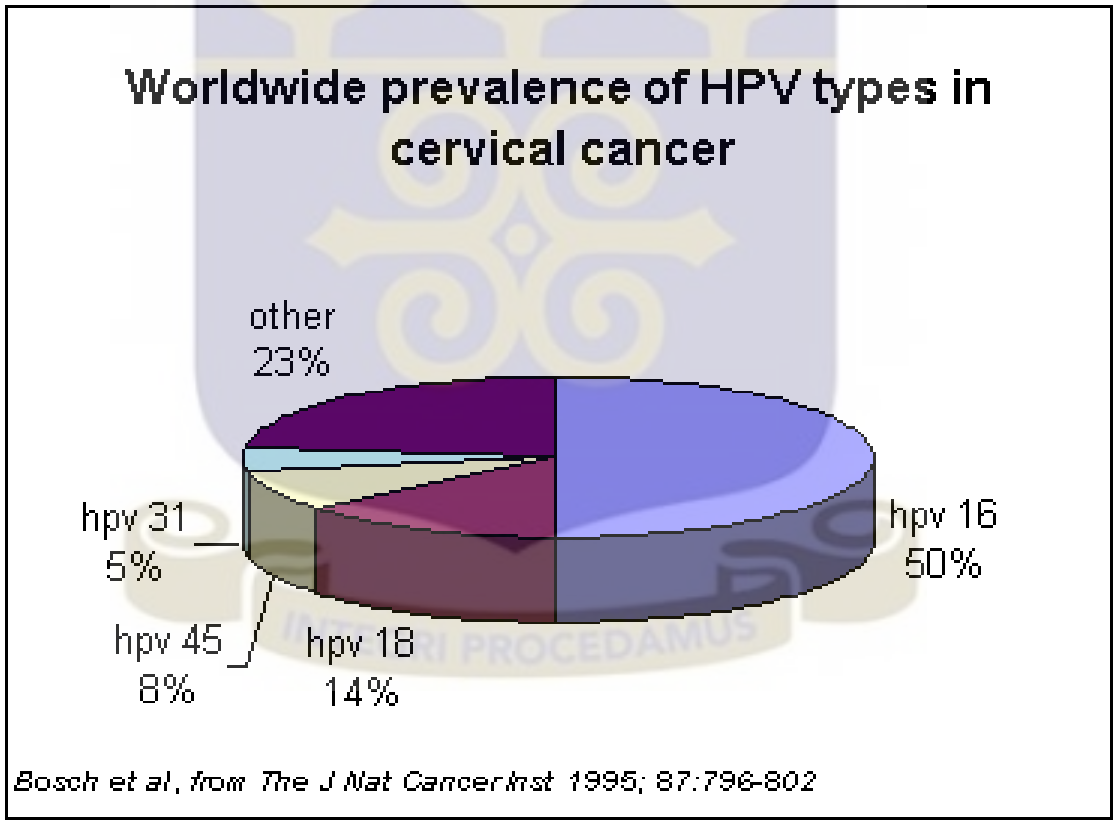


Figure 4: Worldwide Prevalence of HPV types in Cervical Cancer. Adopted by Bosch *et al*, 1995.

2.8 Prevention of HPV Infection and Cervical Cancer

Prevention of genital HPV infection is important in reducing the prevalence of anogenital and cervical cancers. This can be achieved through primary, secondary and tertiary methods. The primary methods include abstinence, condom use and vaccination. There is some evidence that health education programmes that promote abstinence, condom use, or both, could reduce the risk of HPV hence cervical cancer at the population level (Winer *et al*, 2003).

Condom use might reduce the risk for HPV-associated diseases (Manhart & Koutsky, 2002) and mitigate the adverse consequences of infection with HPV. The use of condoms has been associated with higher rates of regression of CIN and clearance of HPV infection in women (Hogenwoning *et al*, 2003) and with regression of HPV-associated penile lesions in men (Bleeker *et al*, 2003). One prospective study among newly sexually active college women demonstrated that consistent condom use was associated with 70% reduction in risk for HPV transmission (Winer *et al*, 2006). However, mutual abstinence until marriage is far from universal, and strict condom use is not completely protective against HPV transmission because the male anogenital skin is not completely covered (Winer *et al*, 2006).

Currently three virus-like particles (VLPs) vaccines produced by recombinant technology (Hagensee *et al*, 1993) have been developed for primary HPV vaccination. These are quadrivalent and nanovalent Gardasil (Merck and Co, Bluebell, PA, USA) and Cervarix (GlaxoSmithKline, Rixensart, Belgium). Gardasil has gained regulatory approval in several countries and Cervarix to date has been approved in 99 countries including Japan, the 27 member states of the European Union (EU), United States, Singapore, Australia, Philippines, Brazil, South Korea, Mexico, Taiwan, Nigeria and Ghana according to a press release from GlaxoSmithKline in October 2009. The two vaccines have been designed to protect against HPV 16 and 18, and Gardasil also protects against low-risk genotypes 6 and 11 and high-risk genotypes 31, 33, 45, 52, 58 (Inglis *et al*, 2006).

In 2013, a pilot project on Gardasil HPV vaccine was introduced in Ghana covering 17 districts in Central, Greater Accra and Northern regions. This is a two-year project supported by Global Alliance for Vaccines and Immunizations (GAVI) Alliance School girls aged 9-11 years were involved in the project (Osei 2013).

Secondary methods of HPV prevention include screening methods such as Pap smear, Visual Inspection with Acetic acid (VIA) or Lugol's iodine (VILI) and HPV genotyping. Pap test screening includes a conventional Pap or a liquid-based cytology (Ferenczy & Franco, 2001). The Pap test, which is the most commonly performed test in developed countries, has been used to reduce cervical cancer significantly. However in developing

countries access to screening is limited. The American College of Obstetricians and Gynaecologists (ACOG), the American Cancer Society (ACS), and the U.S. Preventive Services Task Force (USPSTF) guidelines (2004), state that all women should have a Pap test for cervical cancer screening within 3 years of beginning sexual activity or by age 21 years.

Cervical cancer screening with the Pap test can detect cytologic changes that reflect the underlying tissue changes. However, cytologic abnormalities detected by the Pap test can be ambiguous or equivocal. Abnormalities include ASCUS, atypical glandular cells, low- and high-grade squamous intraepithelial lesions (LSIL and HSIL), and AIS. HPV types 16 and 18 are more commonly found in association with higher-grade lesions. In one study, the prevalence of HPV 16 was 13.3% among ASCUS, 23.6% among LSIL, and 60.7% among HSIL Pap tests (Datta *et al*, 2006).

Although Pap smear remains the most common screening test for cervical cancer, many less developed countries do not have adequate resources to implement cytology-based prevention programs. An alternative, low-cost test, visual inspection using acetic acid (VIA), has been developed for use in low-resource settings where it can be performed by auxiliary health professionals (Soler *et al.*, 2000, ACOG, 2004, Khodakarami *et al.*, 2010**)**.

HPV genotyping is performed by polymerase chain reaction (PCR) with DNA sequencing. Several cross-sectional studies have reported that HPV DNA testing is more sensitive than cytology in detecting high-grade CIN (Mayrandet al, 2007, Arbyn *et al*, 2006). HPV testing provides clinicians with the specific HPV type present in a patient's sample. Knowing a patient's HPV type provides clinicians with valuable information to guide follow up treatment and vaccination. Cervical cancer screening is now entering a new era, in which reliance on measuring the causal viral infection, oncogenic HPV, rather than the pleomorphic cellular changes caused by the infection is increasing (Meijer *et al*, 2006). Periodic screening with HPV tests will provide a useful means to monitor the duration of protection in the population as successive cohorts of women receive HPV vaccination (Meijer *et al*, 2006).

Clinical utility of HPV genotyping will make current screening more programmatically efficient by better allocating resources according to risk. In developing countries, where more than 80% of cervical cancer incidence and mortality occur and where screening programs are either ineffective or non-existent, it is unclear whether HPV genotyping can be made available and would be useful (Meijer *et al*, 2006).

Tertiary prevention of cervical cancer includes diagnosis and treatment of confirmed cases of cancer. Treatment is through surgery, radiotherapy and sometimes chemotherapy. Palliative care is provided to patients when the disease has already

reached an incurable stage. Treating HPV infection is often difficult and frustrating for both the patient and physician. Treatment of visible genital warts for the average patient often requires several treatments before symptoms subside. These treatments are not cures. After treatment the virus may remain in nearby skin and lie dormant for months or even years before becoming visible again, and in some cases visible warts never return. However there is no effective systemic treatment for HPV types that causes cervical cancer (CDC, 2013).

2.9 Risk Factors for HPV Infection

2.9.1 Sexual behaviour

Epidemiological studies investigating risk factors for HPV infection clearly and consistently have shown that the key determinants among women are the number of sexual partners, the age at which sexual intercourse was initiated and the likelihood that at least one of her sexual partners was an HPV carrier as measured by his sexual behaviour traits (Kjaer *et al*, 2001).

Many longitudinal and cross-sectional studies have shown that HPV infection is associated with the number of sex partners in a person's lifetime (Vaccarella *et al*, 2006). In the largest prevalence study, conducted by IARC in over 11 000 women in four continents, the prevalence of HPV among women who had had two or more sexual partners in their lifetime was twice that in women who had had only one partner. Women

whose husbands had had extramarital sexual relationships had a 50% higher HPV positivity rate (Vaccarella *et al*, 2006).

A longitudinal study of virgins who started sexual activity during the study period in a Danish population based cohort study of 100 virgins and 105 monogamous women showed that all women who stayed virginal throughout follow-up were consistently negative for both HPV DNA and HPV 16 serum antibodies at enrolment and at each follow-up visit. Only a fraction of those virgins who initiated sexual activity became positive for HPV DNA or HPV 16 serum antibodies. The most important determinant of HPV DNA acquisition in this study was the number of sexual partners the woman had had between enrolment and the follow-up visit, both among initially virginal women and among initially monogamous women (Kjaer *et al*, 2001).

In another study, 14.3% of women aged 18-25 years with one lifetime sex partner, 22.3% with two lifetime sex partners, and 31.5% with more than three lifetime partners had HPV infection (Manhart *et al*, 2006).

The relationship between number of partners and age at first intercourse with the corresponding detection of cervical and penile HPV DNA in adult women and men was examined among the controls (2,225 women and 1,140 men) of a series of 12 case-

control studies of cervical cancer carried out by the IARC in 10 countries: Algeria, Brazil, Colombia, India, Morocco, Paraguay, Peru, Spain, Thailand and the Philippines. The study showed that, in both sexes, genital HPV DNA detection increased significantly with increasing lifetime number of sexual partners and with decreasing age at first sexual intercourse (Bosch et al, 1996; Mu˜noz *et al*, 1996).

2.9.2 Age

In addition to sexual activity, age is an important determinant of risk of HPV infection. Most cervical cancers arise at the squamocolumnar junction between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix and there are continuous metaplastic changes at this site. The greatest risk of HPV infection coincides with greatest metaplastic activity and this occurs at puberty and first pregnancy and declines after menopause. In a study conducted by de Sanjose *et al*, (2007) the age-specific HPV prevalence is highest in young adults (<35 years) and declines at older ages (35-44 years). With the exception of Asia where rates continued to decrease, the HPV prevalence increases in the age-groups (45–54 years and > 54 years) in all regions. However, cervical cancer is more common in women older than 35 years, suggesting infection at a younger age and slow progression to cancer (WHO, 2010).

In a cross-sectional study of 18,498 women aged 15–74 years from 15 areas in four continents, carried out by the International Agency for Research on Cancer (IARC), age-

standardized HPV prevalence varied more than 10-fold between populations (Franceschi et al, 2006). The shape of the age-specific prevalence curves also varied. An inverse relationship between age and HPV prevalence was found in many, but not all countries. In some of the poorest areas studied, e.g. India and Nigeria, HPV prevalence was high in all age groups (Franceschi *et al*, 2006).

In population-based cohort studies in Guanacaste, Costa Rica, the peak prevalence of HPV infection is seen in women less than 25 years of age and in those aged 55–64 years (Herrero *et al*, 2005). Longitudinal studies have shown a similar bimodal curve for incidence of HPV infection in Colombia (Munõz et al, 2004). In the longitudinal study in Costa Rica, the acquisition of new HPV infections was greatest in young women, whereas persistent infections gradually became more prominent with age (Castle *et al*, 2005).

2.9.3 HIV and other Sexually Transmitted Infections (STIs)

Sexually transmitted infections (STIs) are infections that are spread primarily through person-to-person sexual contact. There are more than 30 different sexually transmissible bacteria, viruses and parasites. The most common conditions they cause are human immunodeficiency virus (HIV) infection, gonorrhoea, chlamydial infection, syphilis, trichomoniasis, chancroid, genital herpes, genital warts and hepatitis B infection.

HIV-infected individuals are at higher risk of HPV infection and persistence, and are infected by a broader range of HPV genotypes (Strickler *et al*, 2005; Rowhani-Rahbar *et al*, 2007). This could probably be explained by the alteration of the immune status in the HIV-infected individual which increases the risk for acquisition of a new HPV infection or reactivation of a latent HPV infection (Schneider 1993).

In a meta-analysis including 5578 HIV-positive women worldwide, 58% of the HIV-infected women with normal cervical cytology had HPV prevalence of 36.3% for any HPV and 11.9% for multiple HPV types (Clifford *et al*, 2006)]. The six most common high-risk HPV types were 16 (4.5%), 58 (3.6%), 18 (3.1%), 52 (2.8%), 31 (2.0%) and 33 (2.0%). The most common low-risk type was HPV53 (4.4%). Simultaneous infection with multiple HPV genotypes is more common in HIV-infected women than in non-HIV infected women (Strickler *et al*, 2003).

Results from the IARC's multicenter study found a 2-fold increased risk in cervical cancer for the presence of antibodies to *Chlamydia trachomatis* (OR = 2.1 (95% CI = 1.1-4.0)) and of antibodies to herpes simplex virus-2 (HSV-2) (OR = 2.19 (95%CI = 1.41-3.40) (Smith *et al*, 2002). *Chlamydia trachomatis* often causes cervicitis, which is a chronic infection of the endocervical cells of the transformation zone. Such inflammation may predispose women to other STDs, including genital HPV infection, by damaging epithelial integrity (Stamm, 1999). Studies suggested that a history of *C. trachomatis*

infection was associated with persistence of oncogenic HPV infections, (Silins *et al*, 2005; Samoff *et al*, 2005) and other studies have shown that persistent HPV infections are necessary for progression to high-grade cervical intraepithelial neoplasia (CIN) and carcinoma (Kjaer *et al*, 2002). HSV-2 infection may act in conjunction with HPV infection to increase the risk of invasive cervical carcinoma (Smith et. al., 2002).

2.9.4 Oral Contraceptives

Patients on oral contraceptives (OC) have a relative risk (RR) of 1.5 for condylomata acuminata which increases to 9-8 after long-term use. (Darling *et al*, 1986). Using Southern blot analysis, current OC use was associated with HPV positivity in patients with normal Pap smears ($p < 0.001$) and patients with reactive atypia ($p = 0.03$) but not in patients with CIN (Lrincz *et al*, 1990). An independent and partly linear association between OC use and HPV detected by consensus primer PCR was seen in female students with an OR of 2.8 after one year OC use and OR of 4.6 after 4 to 5 years of OC use (Ho *et al*, 1995). OC use may have a synergistic effect with HPV since increased risk for cervical cancer was only seen in HPV positive women (Bosch *et al*, 1992).

2.9.5 Cigarette Smoking

History of smoking is associated with an increased risk for condylomata acuminata (RR = 3.7; 95% CI 1.8-7.6) (Daling *et al*, 1986). Elevated concentrations of nicotine and cotinine were found in the cervical mucus of smokers and may transform HPV infected

tissue (Schiffman *et al*, 1987). Smoking acts immunosuppressively on the cervix by decreasing the Langerhans cell population and may alternatively or synergistically, promote the acquisition of HPV infection. A synergistic effect between smoking and HPV has been suggested by a case control study which found a limited effect on cervical cancer risk only in HPV positive women (Herrero *et al*, 1989).

2.9.6 Pregnancy

The influence of pregnancy on HPV detection is controversial although the majority of studies find a higher HPV detection rate during pregnancy: 8% to 20% in non-pregnant and 9% to 35% in pregnant women (Smith, *et al*. 1991). Four out of six studies found an increasing prevalence during pregnancy and three out of four studies describe a decrease of HPV detection post partum. Early age at first birth was an independent risk factor for cervical cancer with an OR of 5.0 (95% CI 1-8, 14.2) for age < 16 years vs. 24 years in a consensus PCR-based case-control study (Bosch, *et al*. 1992). It may be speculated that an active transformation zone, exposed to a high HPV load at the end of pregnancy and to trauma during birth at young age when immunity against HPV is still immature, may be some of the reasons required for the development of cervical neoplasia (Schneider 1993).

CHAPTER THREE

3.0 METHODS

3.1 Study Site

North Tongu District which was initially called Adidome District is one of the 25 districts in the Volta Region. Geographically, it shares borders with Central Tongu to the east, to the west with Asuogyaman District and Dangme West, to the north and south with Adaklu and Ada West Districts respectively. The district covers an area of 1940 sq. Km with an estimated population of 89,777 (2010 population census, Ghana Statistical Service) and that of women is 47,285 (52.7%). Its administrative capital is Battor Dugame.

Figure 5: Map of North Tongu

The district lies within the Tropical Savannah Grassland zone. The Volta River runs from the north to the south of the district roughly, dividing it into two parts with each half lying on the bank of the river. Currently the main socio-economic activities are mainly subsistence farming and petty trading.

The District is divided into four Sub-districts for. These are Battor Area Council, Mepe Area Council, Dussor Area Council and Juapong Town Council. The District has one hospital (Battor Catholic Mission Hospital), six Health Centres, three CHPS Zones and one private clinic.

3.2 Study Population/ Inclusion criteria

Women aged 15years or older and lived in three selected towns (Aveyime, Battor and Mepe) of the district were included in the study. Women who had ever had penetrative sexual intercourse with a male were also included.

3.3 Exclusion criteria

Women who were unable to undergo speculum vaginal examination including virgins and those who had undergone hysterectomy or conisation were excluded. Women who were unable to give consent were also excluded.

3.4 Study Design and Sampling Method

The study was a cross-sectional study. The three villages were selected through balloting and each village was entered through Opinion Leaders. Using probability proportional to size, number of participants was selected from each village. Women who gave consent during each conservative visit were recruited and given appointment for sample collection according to their convenience.

3.5 Sample Size

The sample size was calculated to detect 22% prevalence of HPV (i.e. 21.5% prevalence of HPV among women with normal cytology in West Africa, WHO/ICO Information Centre on HPV and Cervical Cancer, 2009) at 95% confidence interval using the formula;

$N= [z^2 p (1-p)] / d^2$ where,

N= desired sample size population

z= the standard normal deviation (set at 1.96 corresponding to the 95% CI)

p= the proportion in the target population estimated to have the outcome

d= Precision or degree of accuracy desired (set at 0.037)

Using 21.5% prevalence of HPV among women with normal cytology in West Africa, a minimum of 481 sample size was calculated. However, a maximum of 500 subjects were considered in this study.

3.5 Sample Collection

A structured, pretested questionnaire was administered to each consenting woman from the three selected villages in the district. The questionnaire included questions on socioeconomic status, religion, sexual, marital status, reproductive and contraceptive use, and smoking status to assess risk factors for HPV infection. Cervical scrapes were collected by gynaecologists at Battor Catholic Hospital from these women. Samples of exfoliated cells from the ectocervix and endocervix were collected with Aylesbury spatula and smeared on glass slides for Papanicolaou (Pap) test on all the women. In addition, cells left on the spatula after smearing were washed into tubes containing 500µl of DNAgard for HPV-DNA detection and stored at room temperature at the Molecular laboratory of School of Biomedical and Allied Health Sciences.

3.6 HPV-DNA Detection and Genotyping

HPV-DNA detection and identification of the genotypes were carried out by nested multiplex PCR (NMPCR) [Sotlar *et al*., 2004] at the Molecular laboratory of School of Biomedical and Allied Health Sciences. DNA was extracted with a commercial kit (Qiagen Ltd, USA) according to the manufacturer's instructions. A single consensus forward primer (GP-E6-3F) and two consensus back primers (GP-E7-5B and GP-E7-6B) were used for the identification of high-risk genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 and low-risk genotypes 6/11, 42, 43, and 44. Nested amplification of the GP-E6/E7 PCR products with type-specific primers were arranged in multiplex PCR primers of four cocktails each containing four or five primer pairs. The PCR reaction mix of 50µl contained 10X PCR buffer (Promega, USA), 2.5 mM $MgCl_2$, 200µM of each of the four oligonucleotide triphosphates (dNTP) (Promega, USA), 15pmols of each E6/E7 consensus primers and 1.25 units of Taq polymerase enzyme (Promega, USA). Four microlitres (4µl) of DNA extracts were used as template for the amplification reactions using a Techne thermal cycler (Progene, Princetion, NJ, USA). Positive and negative controls and in-house controls were added to each cocktail per run.

The cycling parameters for the first round PCR with E63F/E75B/E76B consensus primers were as follows: 94^{o} C for four minutes (initial denaturation), followed by 40 cycles of 94^{o} C for one minute (denaturation), 40^{o} C for two minutes (annealing), 72^{o} C for two minutes (extension) and a single final elongation step of 72^{o} C for 10 minutes. In

the second round PCR, 1µl of first round PCR product and 15pmols of forward and reverse primers for genotyping were used. The other parameters that were used in the first round PCR mix were the same. The cycling parameters were as follows: 94^{o} C for four minutes followed by 35 cycles of 94^{o} C for 30 seconds, 56^{o} C for 30 seconds, 72^{o} C for 45 seconds and a single final elongation step of 72^{o} C for four minutes (Sotlar *et al*., 2004).

3.7 Analysis of Amplification Products

The amplification products were analysed by gel electrophoresis on 2% agarose gel (Sigma, MO, USA) and stained with 0.5µg/ml ethidium bromide (Bio-Rad Laboratories Incorporated, USA). Ten microlitres of each sample was added to 2µl of orange G (5X) gel loading dye (Qiagen Ltd, USA) for the electrophoresis. Hundred base pair DNA molecular weight marker (Sigma, MO, USA) was run alongside the PCR products. The gel was prepared and electrophoresed in 1X TAE buffer (Bio-Rad Laboratories Incorporated, USA) using a mini gel system at 100 volts for one hour and the gel photographed over UV transilluminator (Bio-Rad Laboratories Incorporated, USA) (Sotlar *et al*., 2004).

3.8 Data Storage and Analysis

All hard copy data was stored in a safety cabinet at the National Public Health and Reference Laboratory. Soft copy data was stored in *Epi info version* and SPSS with codes

and data only used by the investigatory team. Data was analysed using *Epi info version 3.5.1* and SPSS version 16. Exploratory analysis was carried out to obtain descriptive statistics. Categorical variables were compared using Chi square test. Prevalence Odd ratios for HPV infection were calculated. Logistic regression analyses were conducted with the significance level set at 0.05.

3.9 Ethical Issues

Ethics approval was obtained from the Ghana Health Service Ethics Committees of the Ministry of Health and School of Allied Health Science of the College of Health Sciences (University of Ghana) see appendix B. Participants were fully informed about the purpose, procedures, risks, and benefits of participating in this study. Those who agreed to participate signed or thumb printed the informed consent form (appendix A). We also sort consent from the parents or guardians of participants who were under 18years of age. Each participant was assured of confidentiality and protection of their responses and data collected for the purposes of the study.

CHAPTER FOUR

4.0 RESULTS

4.1 Baseline Characteristics of Participants

A total of 500 women with age range 15 to 70 years and a median of 35 years were interviewed between 2009-2013.The majority of the women (29.6%) were in the age group 25-34 years and the least (8.0%) in the age group >54 years (Table 1). Most of the women (69%) had at least primary level education with 31% being illiterate.

More than 70% of the women were married and 16.4% were single as shown in Table 1. Majority of the women (80.8%) earned income less than 100 Ghana cedis (GH¢ 100) and about 30% were either farmers or traders. Sixty-five percent of the women were on the National Health Insurance Scheme with 34% paying their own medical bills or depending on relatives. Ninety-nine percent of the women were Ewes with majority (58.2%) residing in Battor. The lowest number (19.4%) lived in Mepe (Table 1).

Table 1: Baseline Characteristics of Women in North Tongu District, 2013

Characteristics	Frequency	Percentage
Age Group (year)		
<25	98	19.6
25-34	148	29.6
35-44	135	27.0
45-54	79	15.8
>54	40	8.0
Total	500	100
Educational Level		
None	155	31.0
Primary	178	35.6
Secondary	151	30.2
Tertiary	16	3.2
Total	500	100
Ethnicity		
Ga Dangbe	4	0.8
Ewe	495	99.0
Hausa	1	0.2
Total	500	100
Residence		
Aveyime	112	22.4
Battor	291	58.2
Mepe	97	19.4
Total	500	100
Marital Status		
Single	82	16.4
Married	358	71.6
Separated	13	2.6
Divorced	24	4.8
Widowed	23	4.6
Total	500	100
Level Of Income (GH¢)		
< 100	404	80.8
101-250	49	9.8
251-500	10	2.0
>500	9	1.8
NA	28	5.6
Total	500	100

Table 2 shows that, of the women who were married, about 33% were in a polygamous marriage. Majority of the husbands were married to two wives (87.3%) (Table 2). Nine percent of the women knew that, their husbands were having extramarital affair. Age at sexual debut ranged from 10 to 30 years with a median age of 18 years with age less than 18 years being 27.8%. Majority of the women (52.4%) had sex at least once a week.

Table 2: Reproductive/Sexual Characteristics of Women in North Tongu District 2013

Characteristics	Frequency	Percentage
Only Wife of Husband		
Yes	240	(67.0)
No	118	(33.0)
Total	358*	(100)
No of Wives		
2	103	(87.3)
3	15	(12.7)
Total	118#	(100)
Age range at first intercourse		
<18	139	(27.8)
18-19	143	(28.6)
>19	162	(32.4)
Unknown	56	(11.2)
Total	500	(100)

*Total number of married women

#Total number of women whose husband had other wives

About 85% of the women had been pregnant before (Table 2a) with age at first pregnancy ranging from 11 to 37 years, median age 20 years. The number of pregnancies per woman ranged from 1 to 17 with a median of 4. About 43% of the pregnancies resulted in miscarriage/abortion and 14% resulted in stillbirth.

None of the women smoked or had ever smoked tobacco. At the time of the study, 13% of the women were using contraceptive; however, 29.2% admitted to ever using contraceptives for a period between one to four years. Injectibles were the most common contraceptive method ever used (50.7%), followed by oral contraceptives (35.6%) and condoms (8.2%) (Table 2a).

Table 2a: Reproductive/Sexual Characteristics of Women in North Tongu District 2013

Characteristics	Frequency	Percentage
Ever been Pregnant-Parity		
Yes	427	(85.4)
No	73	(14.6)
Total	500	(100)
Age at First Pregnancy		
≤19	135	(31.6)
20-24	232	(54.3)
≥25	60	(14.1)
Total	427!	(100)
No. of Pregnancy		
1	57	(11.4)
2-4	189	(37.8)
≥5	181	(36.2)
Total	427!	(100)
Ever Used Contraceptives		
Yes	146	(29.2)
No	354	(70.8)
Total	500	(100)
Types of Contraceptives Ever Used		
IUCD	8	(5.5)
Condoms	12	(8.2)
Injectables	74	(50.7)
Oral Contraceptive	52	(35.6)
Total	146^	(100)

! Total number of women who had ever been pregnant

^Total number of women who ever used contraceptives

4.2 HPV Infection Rate

The overall HPV infection rate was 47.4% (237/500). HPV age-specific infection rate was highest among women aged >54 years (55%), followed by 15-24 years (50%) and lowest among women aged 25-34 years (42.6%) (Figure 5).

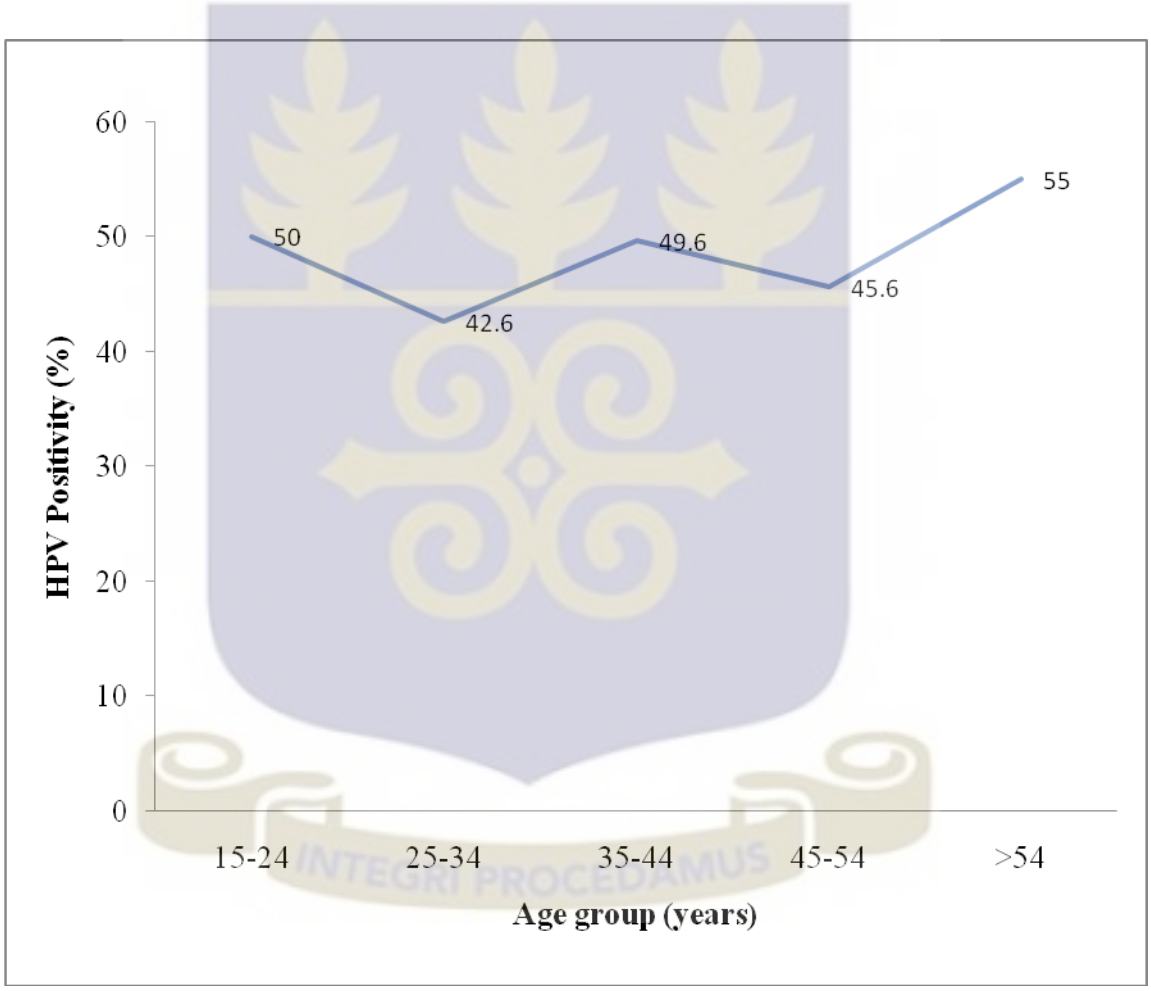


Figure 6: Age-Specific HPV Prevalence among Women in North Tongu, 2009-2013

Table 3 shows that of the HPV positive women, 65% were infected with high risk (HR) types of HPV only, 11.4% with low risk (LR) types of HPV only and 23.6% both HR/LR types of HPV.

Table 3: Risk-type of HPV among Women in North Tongu, 2013

Risk Factors	Proportion of Women Positive for Risk type HPV		
	HR-HPV n(%)	LR-HPV n(%)	HR/LR n(%)
Total	154(65.0)	27(11.4)	56(23.6)
Age Group(Year)			
≤24	32(32.7)	9(9.2)	8(8.2)
25-34	42(28.4)	8(5.4)	13(8.8)
35-44	42(31.1)	5(3.7)	20(14.8)
45-54	22(27.8)	4(5.1)	10(12.7)
>54	16(40.0)	1(2.5)	5(12.5)
Total	154(30.8)	27(5.4)	56(11.2)
Only Wife of Husband			
Yes	67(27.9)	12(5.0)	20(8.3)
No	36(30.5)	6(5.1)	15(12.7)
Total	103(28.8)	18(5.0)	35(9.8)
No of Wives			
2	34(33.0)	4(3.9)	13(12.6)
3	2(13.3)	1(6.7)	2(13.3)
Total	36(30.5)	4(4.2)	15(12.7)
Age range at first intercourse			
<18	33(23.7)	5(3.6)	17(12.2)
18-19	44(30.8)	9(6.3)	15(10.5)
≥20	59(53.1)	7(4.3)	20(12.3)
Total	154(30.8)	27(5.4)	56(11.2)

Table 3a: Risk-type of HPV among Women in North Tongu, 2013

Risk factors	Proportion of Women Positive for Risk type HPV		
	HR-HPV n(%)	LR-HPV n(%)	HR/LR-HPV n(%)
Ever been Pregnant			
Yes	130(30.4)	19(4.4)	50(11.7)
No	24(32.9)	8(11)	6(8.2)
Total	154(30.8)	27(5.4)	56(11.2)
Age at First Pregnancy			
≤19	43(31.9)	2(1.5)	14(110.4)
20-24	65(28)	16(6.9)	27(11.6)
≥25	22(36.7)	1(1.7)	9(15)
Total	154(30.8)	27(5.4)	56(11.2)
No. of Pregnancy			
1-4	64(26.3)	11(4.55)	33(13.6)
5-9	50(34.5)	7(4.8)	14(9.7)
>9	16(41.0)	1(2.6)	3(7.7)
Total	130(30.4)	19(4.4)	50(11.7)
Ever Used Contraceptives			
Yes	38(26.0)	11(7.5)	15(10.3)
No	116(32.8)	16(4.5)	41(11.6)
Total	154(30.8)	27(5.4)	56(11.2)
Types of Contraceptives Ever Used			
IUCD	3(37.5)	1(12.6)	0(0)
Condoms	3(25.0)	1(8.3)	3(25.0)
Injectables	17(23.0)	5(6.8)	9(12.2)
Oral Contraceptive	14(26.9)	4(7.7)	3(5.8)
Total	37(25.3)	11(7.5)	15(10.3)

4.3 Cytology Results

Pap smear was obtained for all the 500 women enrolled, however, cytology results were available for 453 women because the quality of 47 (9.4%) smears was judged to be unsatisfactory giving an unsatisfactory rate of 9.4% (Table 4). Majority (98.9%) of the participants had normal Pap smears. The other with abnormal Pap smears had 0.4% presenting with high-grade SILs, 0.2% ASCUS, 0.2% HPV infection and 0.2% SCC. Of the participants who had normal Pap smears, 77(17%) had bacterial vaginosis, 30(6.7%) had candidiasis 25(5.5%) had atrophy, two had *Shistosoma haematobium* ova in the smears and the same number had *Trichomonas vaginalis infection*. About 9% were satisfactory but interpretation limited by absence of transformation zone or air drying in places (Table 4). Two of the positive women who had ASCUS and HPV infection were aged 21 and 32 years respectively and the rest were in the range of 53-65 years.

Table 4: Association of Cytology Results and HPV Positivity by PCR among women in North Tongu, 2013

Cytology Results	No. cases	HPV Positivity n(%)
ASCUS	1	0(0)
HSIL	2	2(100)
HPV Infection	1	0(0)
SCC	1	1(100)
Negative smear	264	119(45.1)
Negative smear with Atrophy	25	10(40.0)
Negative smear with Bacterial Vaginosis	77	48(62.3)
Negative smear with Candidasis	30	7(23.3)
Negative smear with *Shistosoma haematbium* ova	2	1(50.0)
Negative smear with TV	2	1(50.0)
Satisfactory but limited by absence of TZ component	32	27(84.4)
Satisfactory but limited by air drying	16	10(2)
Unsatisfactory smear	47	11(62.5)
Total	500	237(47.4)

4.4 Cytology and HPV infection

HPVs were detected in the few cases of HSIL and SCC cytology results giving an HPV positivity of 100% (Table 5). However, for ASCUS and HPV infection indication by cytology, no HPV was detected by PCR. Of the 495 negative cytology results and unsatisfactory smears, 234(47.3%) were positive for HPV by PCR.

4.5 Level of Multiple Infections

Majority of the HPV positive women had multiple HPV infections (51.9%). Figure 7 shows that, most of the multiple infections consisted of two different genotypes (27.4%) only and in one case, eight different genotypes were detected.

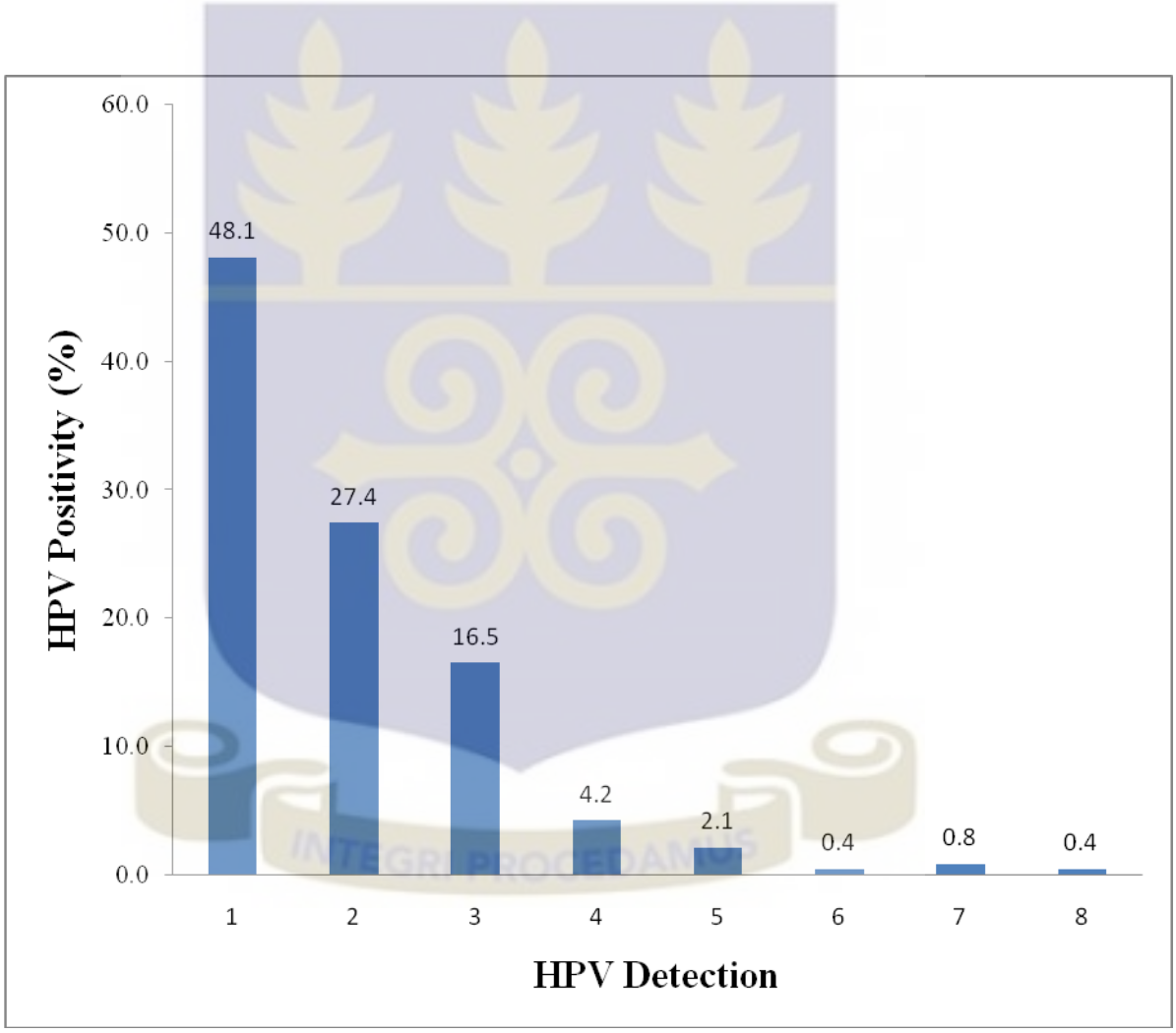


Figure 7: Proportion of Women Positive for one or more HPV Type, North Tongu, 2013

Table 5: Type-specific HPV Positivity among Women in North Tongu, 2013

HPV Type	Single Infection	Multiple Infection	Total HPV Infection	HPV Positive Women(No=237) %	Women in study(N=500) %
HR HPV					
HPV-16	3	1	4	**1.7**	**0.8**
HPV-18	32	23	55	**23.3**	**11**
HPV-31	2	7	9	**3.8**	**1.8**
HPV-33	6	7	13	**5.5**	**2.6**
HPV-35	8	22	30	**12.7**	**6**
HPV-39	2	7	9	**3.8**	**1.8**
HPV-45	2	2	4	**1.7**	**0.8**
HPV-51	3	32	35	**14.8**	**7**
HPV-52	8	57	65	**27.4**	**13**
HPV-56	4	14	18	**7.6**	**3.6**
HPV-58	4	39	43	**18.1**	**8.6**
HPV-59	1	3	4	**1.7**	**0.8**
HPV-66	4	26	30	**12.7**	**6**
HPV-68	8	28	36	**15.2**	**7.2**
LR HPV					
HPV-6/11	3	6	9	**3.8**	**1.8**
HPV-42	2	19	21	**8.9**	**4.2**
HPV-43	21	37	58	**24.5**	**11.6**
HPV-44	1	10	11	**4.6**	**2.2**
Total	114	340	454		

Table 5 shows that single infections were detected in 114 of the women representing 22.8% of the study population and 48.1% of HPV positive women. Of these cases, HPV-18 was the most common HR HPV type (6.4% of study population and 13.5% of HPV positive women) whereas HPV-43 was the most common LR HPV type. Of the multiple infections, the most common HPV type combination was HPV-51 and 52 (13.0%) followed HPV-52 and 58 (8.9%) and HPV-18, 52 and 43 (4.9%).

The 237 HPV-positive women revealed a total of 454 HPV infections with 355 (78.2%) HR HPV types. The most common HR HPV type in either single or multiple infections, was HPV-52, followed by HPV-18, HPV-58, HPV-68, HPV-51, HPV-35, HPV-66, HPV-56, HPV-33, HPV-31, HPV-39, HPV-16, HPV45 and HPV-59. Also the most common LR HPV type in either single or multiple was HPV-43 with HPV-6/11 being the least (Table 5).

Figure 8 shows that multiple HPV infection (two or more HPV types) was common in the age group 35 to 44 years.

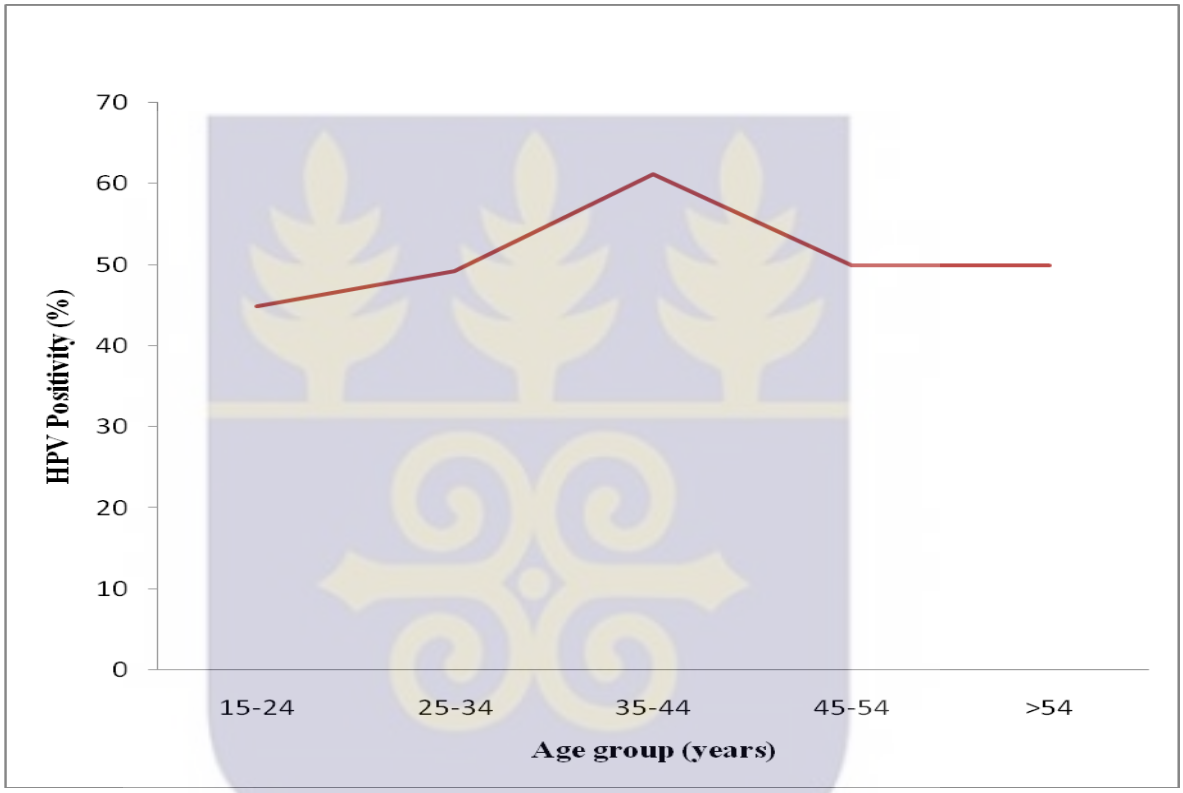


Figure 8: Age-specific Multiple HPV Infection among Women in North Tongu, 2013

Prevalence of Risk Factors for HPV

Table 6 shows that there was no significant association between age and HPV positivity (χ^2=3.0, p=0.57). Of the women who had ever married, 45.5% were HPV positive (χ^2=3.9, p=0.05) and 48.3% of women whose husbands had other wives were HPV

positive. There was also no significant association between age at first debut (($\chi^{2=}$5.7, p=0.13).

Table 6: Univariate Analysis of Participants Characteristics and HPV Infection Rate among Women in North Tongu, 2013

Characteristics	Positive HPV n(%)	Negative HPV n(%)	Total	χ^2(p-Value)
Age group				
<25	49(50)	49(50)	98	3.0(0.57)
25-34	85(42.3)	63(57)	148	
35-44	68(49.6)	67(50.4)	135	
45-54	43(45.6)	36(54.4)	79	
>54	18(55)	22(45)	40	
Ever Married				
Yes	190(45.5)	228(54.5)	418	**3.9(0.049)**
No	47(57.3)	35(42.7)	82	
Only Wife of Husband				
Yes	99(41.3)	141(58.7)	240	1.6(0.21)
No	57(48.3)	61(51.7)	118	
No of Wives				
2	52(50.5)	51(49.5)	103	1.5(0.21)
3	5(33.3)	10(66.7)	15	
Age range at first intercourse				
9-13	6(42.9)	8(57.1)	14	**8.9(0.03)**
14-17	49(39.5)	75(60.5)	139	
18-21	123(47.7)	135(52.3)	143	
>21	31(64.6)	17(35.4)	56	

From table 6a, about 46% of the women who had ever been pregnant were positive for HPV infections. The association between age at first pregnancy and HPV positivity was not significant ((χ^2 = 2.3, p= 0.52). Also of the women who had ever used contraceptives, 43.8% were positive for HPV (Table 6a).

Table 6a: Univariate Analysis of Participants Characteristics and HPV Infection Rate among Women in North Tongu, 2013.

Characteristics	Positive HPV n(%)	Negative HPV n(%)	Total	χ^2(p-Value)
Ever been Pregnant				
Yes	199(46.6)	228(53.4)	427	0.7(0.39)
No	38(52.1)	35(47.9)	73	
No. of Pregnancy				
1-4	108(44.4)	135(55.6)	243	1.1(0.57)
5-9	71(49)	74(51)	145	
>9	20(51.3)	19(48.7)	39	
Age at first Pregnancy				
≤19	59(43.7)	76(56.3)	135	2.3(0.52)
20-24	108(46.6)	124(53.4)	232	
≥25	32(53.3)	28(46.7)	60	
Ever Used Contraceptives				
Yes	64(43.8)	82(56.2)	146	1.1(0.31)
No	173(48.9)	181(51.1)	354	

Table 7 shows that, HPV positivity was not significantly different in the age groups considered. Women aged 25-34 years were less likely of acquiring HPV infection (OR=0.7, 95% CI 0.3-1.5), although this was not significant.

In an unconditional logistic model women, who had never married (being single) were significantly more likely to be infected with HPV infection (OR=1.9, 95% CI 1.03-3.32) (Table 7). Women who had ever been pregnant were about two times more at risk of acquiring HPV (OR=1.6, 95% CI 0.59-4.42), but this was not significant.

Age at first pregnancy was not significantly associated with HPV positivity (Table 7). There was an increasing trend in risk for HPV positivity with increase in parity but the trend was not significant. Women who had given birth to three or four as compared to nulliparous women were two times more at risk than of acquiring HPV (OR=1.6 95% CI 0.64-3.82), although not significant (Table 7).

Table 7: HPV Positivity by Socio-demographic and Reproductive Characteristics among Women in North Tongu, 2013

Risk Factors	HPV Positive no.(%)	OR	95% CI
Age Group			
<25	49(50)	0.92	0.37-2.3
25-34	85(42.3)	0.67	0.3-1.5
35-44	68(49.6)	0.87	0.4-1.9
45-54	43(45.6)	0.71	0.3-1.6
>54	18(55)	1	
Ever Married			
Yes	190(45.5)	1	
No	47(57.3)	**1.85**	**1.03-3.32**
Educational Level			
Illiterate/None	76(49)	1.11	0.73-1.69
Primary/Better	161(46.7)	1	
Ever been Pregnant			
Yes	199(46.6)	1.62	0.59-4.42
No	38(52.1)	1	
Age at First Pregnancy			
≤19	59(43.7)	0.64	0.34-1.23
20-24	108(46.6)	0.76	0.42-1.36
≥25	32(53.3)	1	
No. of Pregnancy			
0	58(52.1)	1	
1-2	50(45)	1.26	0.56-2.79
3-4	49(51.6)	1.56	0.64-3.82
5-6	42(48.3)	1.85	0.73-4.67
>7	38(52.1)	1.62	0.59-4.42

CI-Confidence interval

Women who were the only wives of their husbands were less at risk of acquiring HPV infection (OR=0.18, 95% CI 0.02-1.63), this was also not significant (Table 8). Although there was no statistically significant interaction between HPV infection and number of wives, there was a trend of decreased risk from one to two. There was a significant association between age at first sexual debut and HPV positivity and being in the age group 14-17 years were more at risk (OR=2.8, 95% CI 1.40-5.58). The use of condoms between one to three years was not significantly protective for HPV infection (OR=0.42, 95% CI 0.03-5.86). Women who had ever used IUCD were more at risk than those who had never used it (OR=1.89 95% CI 0.08-44.7) but not significant. Compared to women with normal cytology, women with abnormal cytology were more at risk of acquiring HPV infection (OR=1.7, p=0.44), although this was not significant (Table 8).

Table 8: HPV Positivity by Indicators of Sexual Habits, use of Contraceptive methods and Cytology Results among Women in North Tongu, 2013

Risk Factors	HPV Positive no.(%)	OR	95% CI
Only Wife of Husband			
Yes	99(41.3)	0.18	0.02-1.63
No	57(48.3)	1	
No. of Wives			
1	181(47.5)	8.14	0.68-97.3
2	51(49)	2.06	0.65-6.6
>2	5(33.3)	1	
Age at first intercourse			
9-13	6(42.9)	2.4	0.72-8.10
14-17	49(39.5)	2.8	**1.40-5.58**
18-21	123(47.7)	2.0	**1.06-3.80**
>21	31(64.6)	1	
Ever Used Contraceptives			
Yes	64(43.8)	0.86	0.58-1.29
No	174(48.9)	1	
Types of Contraceptives Ever Used			
Never used	174(49.2)	1	
IUCD	7(87.5)	1.89	0.08-44.7
Condoms	7(58.3)	0.42	0.03-5.86
Injectables	31(41.9)	0.27	0.03-2.83
Oral Contraceptive	18(49.2)	0.17	0.15-1.89
Cytology Results			
Normal	186(46.5)		
Abnormal	3(60)	1.39	0.22-8.86
χ^2 for trend		1.7	p=0.44

CI-Confidence interval

CHAPTER FIVE

5.0 DISCUSSION

5.1 Prevalence of HPV Infection

Compared with women from other parts of the world, women in Africa are least likely to be screened for cervical cancer and have the greatest risk of developing invasive cancer (de Sanjose *et al*, 2007). HPV prevalence in developing countries is higher (15.5%) than in developed countries (10%). Again African women have the highest HPV prevalence (22.1%, 20.9-23.4%) (de Sanjose *et al*, 2007). In Ghana, cervical cancer continues to be a major public health problem, however, there is limited data on the prevalence of HPV (WHO, 2013). The global prevalence of HPV infection in women with normal cytology at any point in time was estimated to be 11.7% in 2010 and 10.4% in 2007 (Bruni *et al*, 2010; de Sanjosé *et al*., 2007; Castellsagué *et al.*, 2007); however, HPV prevalence has been found to vary with a range from 2% to 44% (Bosch and de Sanjose, 2003; Baseman and Koutsky, 2005; Herrero *et al.*, 2005).

In this study, an overall HPV crude prevalence of 47.3% for women with normal cytology was found, which is similar to that reported in other high risk populations in Africa that used PCR-based assays similar to that employed in this study. In these other studies, the prevalence was 44% in Kenya (De Vuyst *et al*., 2003), 40% in rural Mozambique, (Castellsague *et al.*, 2001), 39% in Africans seeking asylum in Puglia, Italy (Chironna *et al*, 2013), 34% in Tanzania (Mayaud *et al*., 2001; Mayaud *et al*., 2003) and

31% in Zimbabwe (Gravittet *et al*., 2002). In urban Nigeria, Senegal, South Africa and rural Nigeria, the prevalence rates were 26.3%, 18%, 15.4% and 14.7% respectively, lower than what was found in this study (Thomas *et al*., 2004; Xi *et al*., 2003; Williamson *et al*., 2002; Gage *et al*, 2012). In Ghana, cervical HPV infection has been reported in 33% and 48% of rural and urban women respectively using dot blot to detect the presence of HPV DNA (Szela *et al.,* 1993). A previous study at the gynaecology outpatient clinic of the Korle-Bu Teaching Hospital (KBTH), Accra, estimated the prevalence of cervical HPV infection in 75 women using the PCR to be 10.7% (Domfeh *et al*, 2008).

These variations may be due to the method of sample selection and/or the type of HPV assay employed. The above studies used either MY09-MY11 or GP5+-GP6+ primers in a conventional PCR which is less sensitive than the novel NMPCR which was used in the current study (Sotlar *et al*., 2004). In Ghana as in many other African countries, polygamy and early age first intercourse are reported in high incidence of HPV infection and cervical cancer (Thomas *et al*, 2004; De Vuyst *et al*, 2003; Parkin *et al*, 2003; Gravitt *et al*, 2002 Castellsagué *et al*, 2001) and this study being consistent with the previous study could explain the high HPV prevalence. Furthermore, according to Bayo *et al*, 2002, factors such as early age at first marriage, marriage with older men or with men that have multiple sexual partners and poor hygienic conditions may explain the high prevalence in African region. It was not possible to evaluate whether the high HPV prevalence observed was be due to HIV infection because presence of HIV infection, a risk factor for high HPV prevalence was not tested for (Strickler *et al*, 2005; Rowhani-

Rahbar *et al*, 2007; Mullick *et al*, 2012). However, HIV prevalence in North Tongu has been increasing since 2009 (i.e. 0.7 in 2009, 1.9 in 2010, 1.5 in 2011 and 2.8 in 2012) (HIV Sentinel Survey Report 2009-2012).

5.2 HPV Type-specific Prevalence

HPV-18 is the second most frequently detected type after HPV-16 globally; however, in Africa, certain HPV types are found more common than in other regions (de Sanjose *et al*, 2007). The prevalence of HPV16 relative to other HPV types varies by region, being highest in Europe and lowest in Africa (Clifford *et al*, 2005) and this study is consistent with the previous study. In Mozambique, in both women with normal cytology and in those with HSIL or worse, HPV-35 was slightly higher than HPV-16 (Castellsagué *et al*, 2001). HPV-52 was the most common HR HPV type in Tanzania (Dartell *et al*, 2012). It was also found slightly more frequently than HPV 16 in Kenya (De Vuyst *et al*, 2003) and in colposcopically normal women in Zimbabwe (Gravitt *et al*, 2002) and this study is consistent with the above study. In Nigeria, the most common LR type was HPV 42. However, HPV-43 was the most common LR type found in this study. Apart from the above HPV type that are common in Africa, HPV types -58, 31, 35, 66, 53, 45, 56 and 33 are also frequently observed (de Sanjose *et al*, 2007) and this was similar in this study where HR HPV types-58, 35 and 66 were also observed in substantial numbers.

The interpretation of variations in HPV type distribution has been attributed to several reasons. As noted by Gravitt *et al* (2002), type-specific HPV prevalence may be influenced by the type of assay used and by the high proportion of multiple HPV infection in certain populations. In this study, majority of the women had multiple HPV infection rate and this may account for the variation. According to Hildesheim and Wang (2002), differences in the relative prevalence of HPV types might be related to the complex geographical and biological interplay between different HPV types or variants and host immunogenetic factors such as HLA polymorphisms.

In a meta-analysis study conducted by Clifford *et al*., (2003) HPV16 was the most common type in SCC (54.3%). The only SCC found in this study was infected by HPV-16.

5.3 Co-infection with Multiple HPV Types

Many epidemiologic studies have indicated that co-infection with multiple HPV types is common. Congruent with previous studies (Richter et al, 2013; De Vuyst *et al*, 2003; Gravitt *et al*, 2002; Castellsague *et al*, 2001), it was found in this study that multiple HPV infections were involved in a considerable fraction of HPV positive women (51.9%). In contrast to studies conducted by Molano *et al*, (2002), prevalence of co-infection with multiple HPV types was 29.7% of the HPV positive women. The majority of the multiple

infections contain two HPV types although in one woman up to eight types was identified.

The high prevalence of multiple HPV infections and high count of types in single patients probably reflects the combination of using a high sensitive PCR assay and case exposure to some risk factors such as early age at first sexual debut. Again in some studies, infections with multiple HPV types have been associated with a higher risk of cervical intraepithelial neoplasia (Sasagawa *et al*, 2001; van der Graaf *et al*, 2002) and in Ghana and other African countries, there is reported high incidence of cervical HPV and cervical cancer infections (Thomas et al, 2004; De Vuyst *et al*, 2003; Parkin et al, 2003; Gravitt *et al*, 2002 Castellsagué *et al*, 2001). However, in other studies, no increased risk of cervical intraepithelial neoplasia or cervical cancer was reported among women with multiple infections compared with women with single HPV infections (Bosch *et al*, 2002). Co-infection with multiple HPV types has also been observed more frequently among younger women (<25 years) (Molano *et al*, 2002; Castellsagué *et al*, 2001). In contrast, co-infection with multiple HPV types in this study was observed more in women aged greater than 35 years.

5.4 Prevalence of Risk Factors

Age pattern of HPV prevalence differs from one country to another, however, the major reported pattern shows an early peak in the young adults (<25 years) just after the start of

sexual intercourse (de Sanjose´ *et al*, 2007; Franceschi *et al*, 2006; Jacobs *et al*., 2000; Kjaer *et al*., 2001), followed by a decline in middle age (35-44 years) after clearance of a large proportion of newly acquired infections and a steady state in the age group 40 years and above (Bosch *et al*, 2008; de Sanjose´ *et al*, 2007; Franceschi *et al*, 2006; de Sanjose´ *et al*, 2003; Matos *et al*, 2003; Shin *et al*, 2003;). In some populations, U-shaped curves with a second peak in postmenopausal women were detected and this observation has generally been found in countries with high incidence rates of cervical cancer (Jacobs et al, 2000; (Herrero *et al*, 2000). In some studies from sub-Saharan Africa, the prevalence of HPV declined significantly with age (De Vuyst *et al*, 2003; Castellsague *et al*, 2001).

Unlike most populations studied so far, HPV prevalence in this study was high not only in women less than 24 years, but also in women with age group 35-44 years and women greater than 54 years. This age pattern was similar to studies in other sub-Saharan African populations like Nigeria and in India (Franceschi *et al*, 2006; Thomas *et al.*, 2004). Furthermore, HPV prevalence remained high or even increased in middle and old age from studies conducted in South Africa, Tanzania, the Gambia and Senegal (Richter *et al*, 2013, Clifford and Franceschi. 2005). The turning point from downward trend to an upward trend was observed after age 54 years consistent with that found in Africa and Europe but not consistent with that found in the Americas which was seen after age 44 years (de Sanjose´ *et al*, 2007). The high prevalence of HPV in all age groups may be a distinctive feature of the populations where HPV transmission continues into middle/old

age and cervical cancer incidence and mortality is very high like Ghana (Wiredu and Armah, 2006). Changes in the sexual behavior of women and their partners throughout their life, resulting in new or re-infection or reactivation of latent HPV infection as the immune system ages may also explain the high HPV prevalence in middle/old age. In this study 11.2% of the women who had ever married reported that their husbands had extramarital relationships and of these 48.9% were HPV positive. Also populations that have very low income levels showed this age pattern (Franceschi *et al*, 2006). This study was conducted in a rural setting with most of the women being either a farmer or trader and earning low income less than Gh¢100.

Being single was a significant risk factor for HPV infection in this study and this correlates with studies conducted in a multinational trial in Ibadan, Nigeria where singles were two times more at risk of acquiring HPV infections (Bahmanyar *et al*, 2012; Thomas *et al*., 2004). This could be explained by the fact that singles tend to have riskier sexual behaviors such as having more than one sexual partner which is risk factor for HPV infection (Bahmanyar *et al*, 2012).

Illiteracy was one of the main correlates of HPV positivity among study women in Ibadan, Nigeria and Accra, Ghana (Thomas *et al*., 2004, Domfeh *et al*, 2008). In contrast to this study, there was no significant association between illiteracy and HPV positivity.

Early age at pregnancy is a risk factor for cervical cancer in developing countries especially those who are highly parous (Louie *et al*, 2009; IARC, 2007; Green *et al*, 2003). In these studies, HPV positivity was high in women who had given birth to three or more children. In this study, although age at first pregnancy was not significantly associated with HPV positivity, there was an increased trend in risk of HPV positivity with increase parity but the trend was not significant. This observation is highly relevant in the African context including Ghana, where young women are engaged in sex at an early age resulting in early pregnancy and therefore high parity. In Ghana and other African countries, there is reported high incidence of cervical HPV and cervical cancer infections (Wiredu and Armah, 2006; Thomas *et al*, 2004; De Vuyst *et al*, 2003; Parkin *et al*, 2003; Gravitt et al, 2002 Castellsagué *et al*, 2001) and this could be the reason for the increase risk in parity. However, it is important to note that in countries with high parity (e.g. in Hunduras), parity among HPV positive cases and controls was not significant (Ferrera *et al*, 2000). According to Bahmanyar *et al*, (2012), the odds ratio for parity suggested a protective effect against HPV infection. Studies conducted by Molano et al (2002), showed decreased risk of HR and multiple HPV infections with increase risk with number of births in contrast to the above studies and this study. In Ibadan Nigeria, parity was not significantly associated with HPV positivity (Thomas *et al*., 2004). In this study, nulliparous compared to women with one or two pregnancies, showed an odds ratio of 1.7 (95% CI 0.7-1.2) in contrast to this study.

Among indicators for sexual activities, early age at sexual debut has been associated with an increased risk of HR HPV infection and in invasive cervical cancer (Bahmanyar *et al* (2012; Louie et al, 2009; Bosch *et al*, 2002). In contrast to the above studies, early age at sexual debut (< 18 years) seemed unrelated to HPV prevalence in Colombia, Nigeria (Molano *et al*, 2002; Thomas et al., 2004). In this study, early age at sexual debut (≤ 18 years) was significantly associated with HPV infection which was consistent with previous studies conducted in Ghana (Domfeh *et al*, 2008).

Intrautrine contraceptive device (IUCD) was the least used in this study in contrast to the Nigerian study (Thomas *et al.*, 2004) and showed an OR of 1.8 (95% CI 0.08-44.7) compared to OR of 1.3 (95% CI 0.9-1.9) in Nigeria. In the Colombian study, IUCD showed an OR of 1.4 (95% CI 0.8-1.5) compared to those who had never used (Molano *et al*, 2002). The use of condom in this study was protective although not significant and this is in contrast to the Accra study (Domfeh *et al*, 2008). However, it was in agreement with the studies conducted by Anh *et al*, 2003 and de Sanjose *et al*, 2003.

Compared with never-users, patients who had used oral contraceptives for fewer than 5 years did not have increased risk of cervical cancer (odds ratio 0.73; 95% CI 0.52-1.03). The odds ratio for use of oral contraceptives was 2.82 (95% CI 1.46-5.42) for 5-9 years, and 4.03 (2.09-8.02) for use for 10 years or longer (Moreno *et al.*, 2002). Women in this study who had ever used oral contraceptives were from one to four years and showed protective effect of use of oral contraceptives but this was not in agreement with the Accra study (Domfeh *et al*, 2008).

In this study we found an HPV detection rate of 46.5% in women with normal cytology and this percentage is similar to that which was found in Southeastern region in Spain (49.7%) (Conesa-Zamora *et al*, 2009). The prevalence of cytology results (1.1%) in this study was consistent with other studies in Ghana (Adadevoh and Forkouh 1993). Cervical intraepitherial neoplsia has also been shown in other studies to be related to advanced age, high parity and rural residence and women with lower socio-economic status.

5.5 Limitation

The sample selection was not based on the population structure of the study area and thus even though the sample size was higher than calculated, some age groups could have been under- or over-represented. There could also be potential response and recall biases. Sexual behaviour characteristic of the participants' male sexual partners were also not determined.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

We found a high HPV prevalence (47.3%) among women in North Tongu. The five most frequent HR HPV types among all women being HPV-52, HPV-18, HPV-58, HPV-68 and HPV-51 in descending order, but HPV-16 was detected in the only SCC found. HPV-43 was the most frequent LR HPV and HPV-6/11 the least. Of the 495 cytology results that were negative, satisfactory or unsatisfactory, 47.3% were positive for HPV infections. Multiple HPV infection was seen in 51.9% and most of it consisted of two different genotypes, although in one woman, eight different genotypes were detected. Of the multiple HPV types, the most common combination was HPV-51, 52, followed by HPV-52, 58 and HPV-18, 52, 43. In all the risk factors assessed, there was significant association between marital status and early age at first sexual debut and HPV infection.

6.2 Recommendation

1. The North Tongu District should increase HPV and cervical cancer education in their public health activities.

2. The high HPV prevalence determined in this study population underscores the urgent need for the Ghana Health Service to implement a national cervical cancer

screening and prevention programme to prevent a marked increase in the incidence of cervical cancer in the country.

3. Ghana Health Service should monitor HPV types because of the high non-vaccine HR HPV when routine HPV vaccination is rolled out through screening.

4. Follow-up data are needed to clarify to what extent the HPV prevalence in middle- and older-aged women is related to the persistence or reactivation of old infections, acquisition of new infections, or cohort effects.

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APPENDICES

Appendix A: Questionnaire

Molecular Epidemiology of Human Papillomavirus Infection of the Cervix in Women in North Tongu District, Volta Region, Ghana

Subject No…… Date……..

Personal Profile

1. Name……..
2. Age……
3. Educational Level: None ☐ Primary ☐Secondary ☐Tertiary ☐
4. Occupation……
5. Residence……..
6. Region……
7. Ethnicity……
8. Marital status: Single ☐ Married ☐Separated ☐Divorced ☐Widowed ☐
9. Level of income: <GH¢ 100 ☐GH¢ 100-250 ☐H¢ 250-500 > GH¢ 500 ☐
10. How do you pay your medical bills? Self ☐latives ☐mployer NH☐S ☐

Risk Factors for HPV infection

Sexual Partners

11. Are you the only wife of your husband? Yes ☐No ☐
12. If no how many wives does your husband have? .....
13. How many permanent sexual partners do you have? .....
14. How many casual sexual partners do you have? .....
15. Does your husband have extramarital sexual relationships? Yes ☐No ☐Unknown ☐
16. At what age did you first had sex? .....
17. How often do you have sex? .....

History on Pregnancy

18. Have you ever been pregnant? Yes ☐ No ☐

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

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

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

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

Smoking status

19. Do you smoke tobacco? Yes ☐ No ☐
    If yes, how long have you been smoking? .....
    How many cigarettes on average do you smoke per week? .....

20. Have you smoked before? Yes ☐ No ☐
21. If yes how long did you smoke? .....
22. Do you use any other tobacco-related product? (snuff) Yes ☐ No ☐
    If yes, how long have you been using it? .....
    How many tobacco-related products on average do you smoke per week? .....

Contraceptive Use

23. Do you currently use any form of contraception? Yes ☐ No ☐

If yes which of these do you use? IUCD ☐ Condoms ☐ Injectable progestogen ☐

Combined oral contraception ☐

Other specify.....

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

Less than 3 months ☐ Between 3 months and 1 year ☐

Between 1 year and 5 years ☐ More than 5 years ☐

25. If not currently on contraception, have ever used any form of contraception?

Yes ☐ No ☐

If yes specify IUCD ☐ Condoms ☐ Injectable progestogen ☐

Combined oral contraception ☐

Other Sexually Transmitted Infection

26. What is your HIV status

Unknown ☐ Negative ☐ Positive ☐

If positive, when was it diagnosed? .....

27. Are you using anti-retroviral therapy? Yes ☐ No ☐
28. Do you have any other sexually infected infection? Yes ☐ No ☐

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

When was it diagnosed? .....

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

Yes ☐ No ☐

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

When was it diagnosed? .....

Subject number: ……..

CONSENT TO PARTICIPATE IN A RESEARCH PROJECT

TITLE OF PROJECT: Prevalence and Distribution of Human Papillomavirus Genotypes Among Women in North Tongu District, Volta Region, Ghana

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

Explanation of Procedures

You are being invited to participate in a research project to determine the prevalence and genotypes of HPV infection in women in the North Tongu District. The approach of the research is through the use of a questionnaire and cervical specimen collection. You will complete the questionnaire that will require answers on personal profile and risk factors for HPV infection (i.e. number of sexual partners, smoking status, contraceptive use etc.). Afterwards, you will be examined by medical officer and cervical specimen will be collected.

Risks and Discomforts

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

Benefits
There are no direct benefits by participating in this project. However, this research is expected to provide data on HPV genotypes and distribution for policy makers when vaccination becomes a mainstream policy in Ghana.

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

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

Costs and/or Payments to Subject for Participation in Research
There will be no costs for participating in the research. Also, you will not be paid to participate in this research project.

Any questions concerning the research project and/or in the case of injury due to the project, participants can call Professor E. K Wiredu, School of Allied health Sciences (0244664184), Miss Gifty Boateng of the School of Public Heath (0277-456-019) or Dr. Kofi Effah of Battor Catholic Hospital (0244271027).

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

Consent to participate in Research

I, ……

□ Confirm that I have read the written information (or have had the information read to me) for the study **Prevalence and Distribution of Human Papillomavirus Genotypes Among Women in North Tongu District, Volta Region, Ghana: Implications for Use of Current HPV Vaccines** and that the study procedures have been explained to me by study staff during the consent process for this study.
□ Confirm that I have had the opportunity to ask questions about this study and I am satisfied with the answers and explanations that have been provided.
□ Understand that I grant access to data to authorised persons described in the information sheet.
□ Have been given time and opportunity to consider taking part in this study.

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

I consent to participate in the above research study.

Signature of Subject: .....
Date.....

Signature of Interviewer: .....
Date.....

Name of
Impartial Witness: ……

Signature of
Impartial Witness: ……
Date.....

Thumb print of subject unable to sign

Appendix B: Ethical Clearance

GHS Research Ethics Committee
Report on Expedited Review

Name of Principal Investigator(s) Gifty Boateng

Project Title: Prevalence and distribution of Human Papillomavirus Genotype among women in North Tongu District, Volta Region, Ghana.

Technical/Scientific Issues of Concern including suggestions for improving design.

One specific objective is to determine the risk factor for HPV infection but there is no description of how this will be done in the document.

Ethical issues of concern with suggestions for modification

Addressed appropriately

Decision

1. Recommended for Ethical Approval
2. Recommended for Ethical Approval after modification
3. Ethical approval cannot be granted in its current form.
4. Other (specify)..... NM

Signature of reviewer..... **Date** 24/4/10

Name of Review.....