SCHOOL OF PUBLIC HEALTH

COLLEGE OF HEALTH SCIENCES

UNIVERSITY OF GHANA

PREVALENCE OF HUMAN PAPILLOMAVIRUS AMONG WOMEN ATTENDING RIDGE HOSPITAL, ACCRA

BY

ALABIRA IDDRISU ALHASSAN

(10396531)

THIS DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PUBLIC HEALTH DEGREE

JULY 2013

DECLARATION

I do hereby declare that this work is the results of my own research and has not been presented by anyone for any academic award in this or any other university. All references used in the work have been fully acknowledged.

I bear sole responsibility for any shortcomings.

…….. ……

ALABIRA IDDRISU ALHASSAN DATE

… …… …….

DR FRANCIS ANTO DATE

SUPERVISOR

DEDICATION

This work is dedicated to Almighty God for His mercy, love, guidance and protection throughout this program.

I also dedicate this work to my late mother Alabira Adamu for her support and encouragement. May she rest in perfect peace.

ACKNOWLEDGEMENT

I thank Almighty God for the guidance and strength He bestowed on me throughout the course.

I wish to express my sincere gratitude to my candid and supporting supervisor, DR. Francis Anto for his timeless dedication, guidance, corrections and untiring efforts towards the achievement of this work. My sincere gratitude also goes to Prof. Isabella A. Quaye (Former Dean, SPH) for the motherly love and encouragement towards the achievement of this work. I sincerely thank Dr. Priscilla Nortey for her support and encouragement. I also thank Dr. Emmanuel Srofenyoh, Head of clinical services, Ridge Hospital, for the opportunity and support give me. My sincere gratitude also goes to Sisters Mavis Apatu (Head of Family planning Unit, Ridge Hospiatl), Juliet Amawu and Doris Agyei of Ridge Hospital for their immense contribution towards the collection of the samples for this research work. I sincerely appreciate the support given to me by Dr Kwame Aryea, University of Ghana Medical School, and Dr Davis E. Aduamah of Korlebu Teaching Hospital for their assistance. My sincere gratitude also goes to Dr Richard Harry Asmah, School of Allied Health Sciences and my personal assistant Patricia Anum for the immense support and encouragement given to me during the laboratory analysis. I also want to thank Mrs Mary Baaba Crentsil (SPH Library) for the patience and support shown to me during my stay in the school. My sincere gratitude goes to the women who agreed to take part in the study by providing me their personal information and also allowed me use their samples for laboratory analysis. Finally, I am highly indebted to my wife Linda Sandow, all family members and friends for their financial and material support throughout my study and particularly towards this thesis.

ABSTRACT

In Ghana, cervical cancer constitutes about 57.8% of all gynaecological cancers. It is the second most common cancer in women with an estimated incidence of 26.4 per 100,000. HPVs are aetiological agents of cervical cancer. Genital HPV are the most common sexually transmitted viruses in human.

The objective of the study was to determine the prevalence and genotypes distribution of HPV 6, 11, 16 and18 among women attending the Family Planning and Human Papilllomavirus screening centre of the Ridge Hospital.

It was a quantitative cross- sectional study on the prevalence of HPV infection among women aged 19 to 60 years attending the Family Planning and HPV Screening Centre of Ridge Hospital. 201 ecto and endocervical samples were taken for the extraction of HPV-DNA and detection of HPV 6, 11, 16 and 18 using polymerase chain reaction. HPV dual priming oligonucleotide primers, which was a multiplex system for HPV genotypes, was used for amplification. A data analysis was done using Stata 11 to determined odds ratios and the prevalence of HPV among the women.

The mean age of participants was 38.43 ± 11.81 years and the percentage of lifetime single sexual partner was 26.37%. The HPV prevalence was 15.92%. Of the women with HPV 6, 11, 16, 18 or other types detected, 98.51 % had one type, 1.49% had two types. Overall prevalence of specific types was 1.00% for HPV 6/11, 2.99% for type 16, and 1.99% for type18 and 9.95% for other types.

In conclusion, this study suggests a statistically significant association between having multiple partners and the prevalence of HPV infection (OR=2.68, p=0.01). Vaccination against HPV should be made available and accessible to Ghanaian girls before age 18 years.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMS

8- Mop	-	Methoxypsoralen
CIN	-	Cervical Intraepithelial Neoplasia
CIS	-	Casinoma In Situ
FPHC	-	Family Planning And HPV Screening Centre
HPV	-	Human Paillomavirus
HRC	-	High Risk Common
HR-HPV	-	High Risk HPV
ICC	-	Invasive Cervical Cancer
LR-HPV	-	Low Risk HPV
PCR	-	Polymerase Chain Reaction
SIL	-	Squamous Intraepithelial Lesions
STD	-	Sexual Transmitted Disease
STI	-	Sexual Transmitted Infection
VIA	-	Visual Inspection With Acetic Acid
WHO	-	World Health Organization

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Background

Human papillomaviruses (HPVs) are aetiological agents of cervical intraepithelial neoplasia and cervical cancer. Genital HPVs are the most common sexually transmitted viruses in human. Human papillomaviruses (HPVs) have been classified into high risk types (HR-HPV) and low risk types (LR-HPV). The high risk types have been associated with invasive cervical cancer and its cytological precursors, squamous intraepithelial lesions. HPV 16 is the most common HPV that is detected in about 50% of cervical cancer cases (Domfeh et al, 2008). Human papillomaviruses infection causes about a 100% of cervical cancer cases and is associated with the incidence of other cancers including vulva cancer (WHO, 2008b).

Over 80% of the yearly 500,000 new cervical cancer cases and 280,000 cervical cancer deaths worldwide occur in developing countries. The highest burden and mortality associated with cervical cancer worldwide is in Sub-Saharan Africa (Edwin, 2010 and Okonofua, 2007).

In Ghana, cervical cancer constitutes about 57.8% of all gynaecological cancers. It is the second most common cancer in women with an estimated incidence of 26.4 per 100,000. It is also the second most common cancer in women aged 15 to 44 years in Ghana. Every year, 3,038 women are diagnosed with cervical cancer and 2,006 die from it in Ghana (Edwin, 2010; Nkyekyer, 2000; WHO, 2010).

Prevalence of genital HPV is directly related to the number of lifetime sexual partners, recent change in sexual partners, marital status, age at first sex, illiteracy, oral contraceptive use, alcoholism, hormonal and dietary factors and immune suppression (Domfeh et al, 2008).

Cervical cancer screening has been vibrant and effective in reducing morbidity and mortality rates in the developed countries for several decades now. This has not been the case in developing countries especially Sub Saharan African countries (Edwin, 2010).

Several screening methods have been adopted to combat the spread of HPV. These methods are Papanicolaou's smear (Pap smear), visual inspection with acetic acid (VIA) and HPV-DNA using polymerase chain reactions. The Pap smear and VIA have been observed to have challenges with regards to sensitivity and specificity even though the Pap smear is the gold standard for screening in Ghana. Polymerase chain reaction (PCR) involves the use of HPV-DNA in the screening of HPV and cervical cancers and this method has good sensitivity and specificity. This study used the method of PCR in the screening of HPV among women attending Ridge Hospital Family Planning and HPV screening Centre.

The World Health Organization (WHO) has underscored the importance of screening, stating that the introduction of HPV vaccine should not undermine or divert funding from effective screening programmes for cervical cancer (WHO, 2009).

1.2 Problem Statement

Cervical cancer is the second most common malignant neoplasia affecting women worldwide with about 86% of cases in developing countries; where the disease accounts for 15% of all cancers in women. Less than 50% of women diagnosed of cervical cancer in this part of the world survive for longer than five years. In Ghana, cervical cancer is the second most common cancer in women and constitutes 57.8% of all gynaecological cancers.

Each year 60-70% of all cervical cancer cases worldwide are the result of high risk HPV types 16 and 18, the oncogenic genotypes. In West Africa, cervical cancer rates are among the highest in the world, with an age standardised incidence rate of 33.7 per 100,000 women and that of Ghana is 39.5 per 100,000 women. Due to lack of cytology-screening and early treatment programmes, the age standardised mortality rate among women diagnosed with cervical cancer is 27.6 per 100,000 or 2,006 deaths per year in Ghana, slightly higher than that of the sub-region which is 24.0 per 100,000.

Efficacious vaccines that provide protection against high risk HPV genotypes and reduce the risk of developing cervical cancer and associated morbidities and mortalities are available. These vaccines are especially valuable in resource limited settings like Ghana where secondary prevention methods such as cytology screening are not widely available. Like most other vaccines, current HPV vaccines are genotype specific. At the moment, very limited data are available on the common genotypes of HPV circulating among women in Ghana. Some more data are therefore needed in order to establish which vaccine is more appropriate for use in the country.

1.3 Objectives of the Study

General Objective

To determine the prevalence and genotypes distribution of Human Papillomavirus (6, 11, 16 and18) among women attending the Family Planning and Human Papilllomavirus screening centre (FPHC) of the Ridge Hospital.

Specific Objectives

1. To determine the prevalence of HPV among women attending Family Planning and HPV screening centre.
2. To determine the age specific distribution of HPV among women attending FPHC.
3. To determine the distribution of HPV 6, 11, 16 and 18 among women attending FPHC.

1.4 Research Questions

The research questions of the study are:

1. What is the prevalence of HPV 6, 11, 16 and 18 among women attending the FPHC of Ridge Hospital?
2. What is the age distribution of HPV 6, 11, 16 and 18 among the women?

1.5 Conceptual Framework

Human Papillomavirus is acquired through sexual contact. The sexual history, sociodemographic characteristics, gynaecological and medical history influence the acquisition of HPV and the prevalence of HPV 6, 11, 16 and 18 among women.

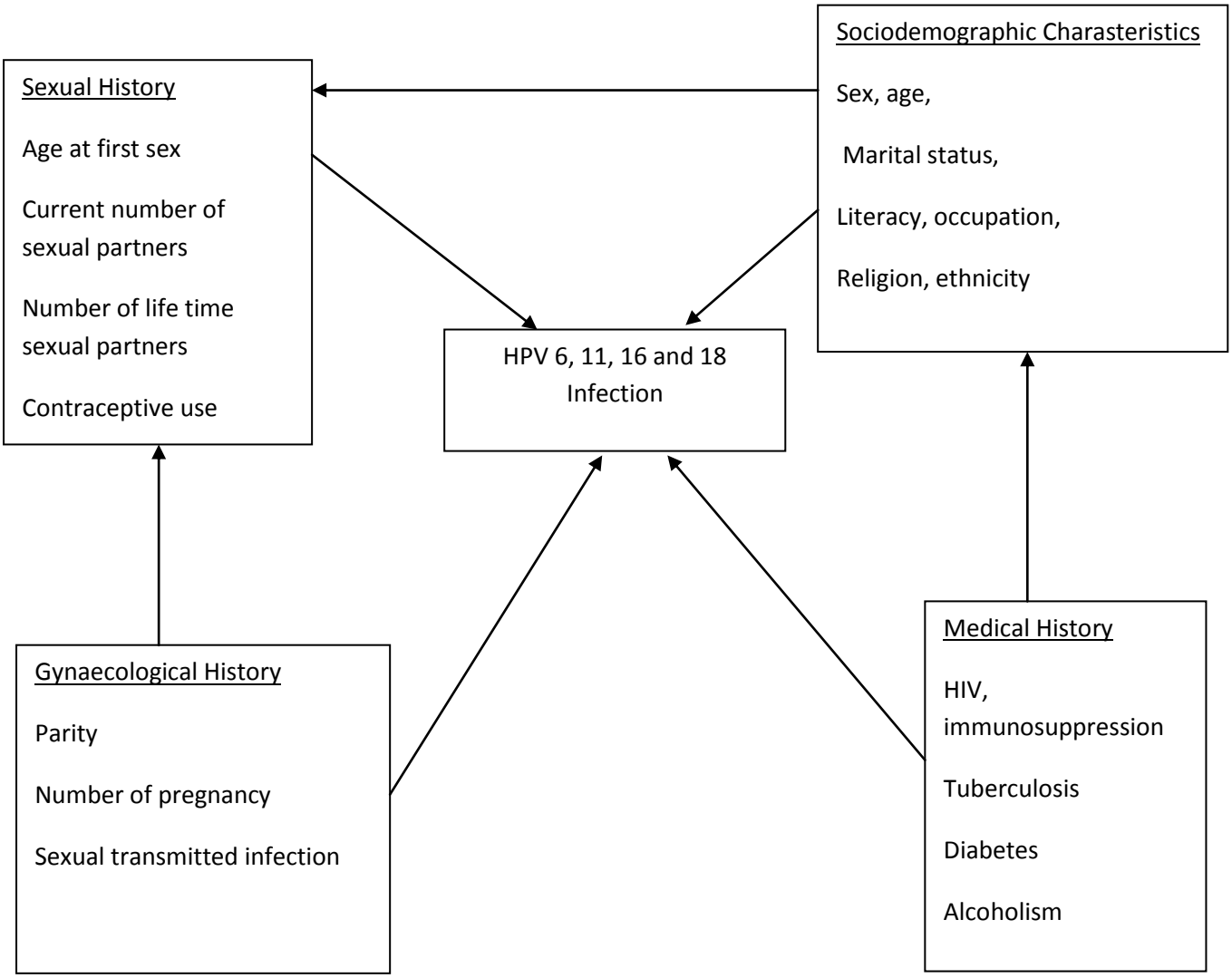

Figure 1: Framework of Factors Affecting the Prevalence of Human Paillomavirus

1.6 Justification of the Study

In 2006 a quadrivalent HPV vaccine against both cervical cancer precursors and external genital lesions caused by HPV types 6, 11, 16 and 18 was licensed for use in the United States. Fortunately, Ghana has introduced a bivalent vaccine targeting HPV 16and 18 this year (2013) which is a major milestone to reduce the impact of cervical cancer. Although this is expected to provide excellent protection against cervical cancer caused by HPV16 and18, it cannot provide adequate protection against other genotypes such as HPV 6 and 11.

Ghana introduced Papanicolaou (Pap) smear in an attempt to eliminate cervical cancer. Unfortunately, the introduction of Papanicolaou (Pap) smear has challenges with regards to sensitivity and reliability. Also, it could not determine the HPV genotypes that are responsible for cervical cancer in Ghana. Therefore, there is the need to look at a more sensitive method such as molecular based methods.

The use of more sensitive molecular methods for detecting HPV genotypes will help establish the specific genotypes that are common in Ghana. It will also provide the basis for vaccination-based strategies for the primary prevention of cervical cancer.

CHAPTER TWO

LITERATURE REVIEW

2.1 Virology of Human Papillomavirus

Human Papillomaviruses are small non-enveloped double stranded DNA viruses. Their life cycles take place entirely and only within stratified squamous epithelial cells. They first bind to the basement membrane at sites of trauma in the epithelium to initiate infection. The infected basal cells then migrate into the wound. Heparan sulfate proteoglycans is the initial cell surface attachment factor. The infectious process takes 2-3 days to initiate viral gene expression. After cell surface binding, determinants of L1, the major capsid protein, route the virions to late endosomes, where uncoating occurs. L2, the minor capsid protein is then required for escape from the endosome. A complex is formed between L2 and viral genome. This L2/viral genome complex interacts with a sub nuclear domain designated ND10 in the nucleus to initiate viral gene transcription. Viral gene expression in the basal cells is at a low level and limited to the non-structural early genes, particularly E1 and E2, which are involved in autonomous replication and transcription of the viral genome. High level of both early and late viral gene transcription occurs in response to the induction of terminal differentiation in the intermediate and upper layers of the epithelium. This leads to viral genome amplification. The function of E6 and E7 oncogenes is to induce replication of the viral genomes in the differentiating keratinocytes that are not replicating their own DNA. The genomes are then encapsidated in L1/L2 particles and the virions are released in the desquamating keratinocytes (Shciller, 2008).

More than 100 HPV types have been identified, and about 40 can infect the genital tract. These HPVs are classified in to high risk types (HR-HPV) and the low risk types (LR-HPV). The HR-HPVs are associated with anogenital cancers whiles the LR-HPVs are associated with genital warts. It is accepted that all squamous cell cervical carcinomas contain at least

one of the 18 HPV considered oncogenic high-risk types. HPV 16, 18, 31, 52 and 58 cause 50% of the HPV infection .The association is supported by strong epidemiological evidence and the detection of HPV DNA in up to 99.7% of cervical cancers from all geographic areas (Garcia-Espinosa et al., 2009).

2.2 Epidemiology of Human Papillomavirus, Cervical Cancer and Genital Warts

Clinical and subclinical human papillomavirus infections are the most common sexually transmitted infections in the world. The prevalence of genital HPV infection among asymptomatic women in the population is between 2% and 44%.The age-standardized prevalence rates of HPV infection in European, Asian, South American, and sub-Saharan African women without cytological abnormalities were estimated at 5.3%, 8.7%, 14.3%, and 25.6%, respectively. A recent meta- analysis estimated HPV prevalence among women with normal cytology using data from 78 published studies. The adjusted global prevalence was 10.4% with considerable variation by region. About 291 million women worldwide are harbouring HPV and around 105 million women worldwide have HPV 16 and 18, the most common oncogenic types in cervical carcinoma (Ann et al.,2006).

The prevalence of HPV is highest among young women soon after the onset of sexual activity and falls gradually with age, possibly as a reflection of accrued immunity and a decrease in the number of sexual partners (Trottier & Franco, 2006) .

In some populations, however, the age-specific prevalence curve rises again at ages 45 or 50 years coinciding with the peri-menopausal or immediately after postmenopausal years. The reason for this second peak is unclear, but it could be related to one or more non mutually exclusive mechanisms, such as reactivation of previously undetectable infections acquired earlier in life (due to a gradual loss of type-specific immunity or a sudden loss via hormonal influences during the postmenopausal years); acquisition of new infections due to sexual contact with new partners later in life (Trottier & Franco, 2006).

HPV 16 is the most prevalent HR-HPV, and is present in about 54% of cervical cancer specimens worldwide, whereas HPV-18 is associated with approximately 17% of cervical cancers. The remaining cancers have been shown to contain DNA from other high risk types, such as HPV-45, -31, and -33. It is now widely accepted that HR-HPV infections are necessary, but not sufficient, cause of virtually all cases of cervical cancer worldwide (Trottier & Franco, 2006). Among the low risk group, HPV 6 and 11 cause majority of clinical diseases. They dominate by virtue of prevalence of genital warts. HPV 6 and 11 are rarely associated with various malignancies including vulval and penile cancers (Lacey et al, 2006).

Studies among women before and after their first sex strongly support that HPV is sexually transmittable. A number of cohort studies were done on women who were virgin and initially free from HPV. These women were followed into their sex life. The results showed the incidence rate of HPV among these women to be between 14% and 36% women years. This translates to a high infection rate from the time of first sexual intercourse. Moreover, co-infection with multiple HPV types is a common finding of many epidemiologic studies involving women after three years of sexual debut. About 75% of sexually active people have HPV exposure in their life time (Trottier & Franco, 2006).

Epidemiology of Cervical Cancer

Cervical cancer is the second most common malignant neoplasm affecting women worldwide with about 86% in developing countries. Less than 50% of women affected by cervical cancer in developing countries survive longer than 5 years. In 2008, an estimated 529,409 new cases and 274,883 deaths from cervical cancer occurred globally. Cervical cancers account for 15% of female cancers in developing countries and about 3.6% in developed countries. Globally, the ratio of mortality to incidence is 55% with poor survival rate in developing countries and good survival rate in the developed countries (WHO, 2010).

Cervical cancer is seen as an important cause of lost years because it affects young women. It is responsible for 2.7 million years of lost life worldwide (Parkin & Bray, 2006).

In Ghana, cervical cancer constitutes about 57.8% of all gynaecological cancers. It is the second most common cancer in women with an estimated incidence of 26.4 per 100,000. It is also the second most common cancer in women aged 15 to 44 years in Ghana. Every year, 3,038 women are diagnosed with cervical cancer and 2,006 die from it in Ghana (Edwin, 2010; Nkyekyer, 2000 ; WHO, 2010).

Epidemiology of Genital Warts

A number of epidemiological studies have examined the association between genital warts and age, sexual history and socio demographic factors. These researches have indicated a strong association between genital warts and some of these factors. It has been shown that genital warts are widespread in the urban areas. The disease is also seen to be very high among young girls age 15 to 24, who are sexually active. This age group is involved in the ongoing transmission of HPV6 and 11. This is partly due to increased number of sexual partners. There is an increased prevalence of genital warts among women who have HIV due to their immune status (Lacey et al, 2006).

Genital warts are a global public health problem with increased prevalence annually in both developed and developing countries. Health Protection Agency in United Kingdom recorded 79,618 new cases of genital warts in 2004. National Disease and Therapeutic Index in United States recorded 316,000 new cases of genital warts in 2004. Existing data suggest that almost the same rate of new cases are reported in many countries even though some of the countries do not have formalised systems of gathering data on genital warts (Lancey et al, 2006)

2.3 Cervical Cancer Clinical Ramifications

The development of cervical cancer begins as a slow process of disruption of the normal maturation of the transformation zone epithelium of the uterine cervix near its squamo-columnar junction. This process of abnormal changes is initially limited to the cervical epithelium. These pre invasive lesions, known as dysplasia (or as cervical intraepithelial neoplasia (CIN) or as squamous intraepithelial lesion (SIL), are invariably asymptomatic and can be discovered only through cytological examination using the Papanicolaou (Pap) smear and confirmed by coloscopic examination and biopsy.

If left untreated, with persistent infection the low grade lesions may eventually extend to the full thickness of the cervical epithelium (cervical carcinoma in situ [CIS]) and traverse the lining formed by the basement membrane to become invasive. This process may take a decade or longer, mostly twenty years, but will finally occur in a large proportion of patients with CIS.

There may be inflammation at the site of primary HPV infection and HPV is capable of suppressing the host immune response. Cell-mediated immune responses are important in controlling both HPV infections and HPV-associated neoplasms as shown by the increased prevalence of these diseases in people with immune suppression. Invasive cancer will have its lesion grow unconstrained and reach small blood and lymphatic vessels to finally become metastatic in body sites (Trottier & Franco, 2006).

The major steps known to be necessary in cervical carcinogenesis include HR-HPV infection, persistence of that infection over a certain period of time, progression to precancerous lesions, and, eventually, invasion. HPV infects the stratified squamous epithelium and stimulates cellular proliferation. Infected cells display a wide range of alterations, from benign hyperplasia to dysplasia to invasive neoplasia. It is only invasive neoplasia that is not reversible all the other steps in the process leading to the invasive stage are reversible, including clearance of HPV infection and regression of precancer, which happens in many

women who experience HPV infection. Majority of HPV infections are transient, with only a small proportion becoming persistent (Trottier & Franco, 2006).

2.4 Symptoms and Signs of Cervical Cancer

Women with early cervical cancers and pre-cancers usually have no symptoms. Symptoms often do not begin until a pre-cancer becomes a true invasive cancer and grows into nearby tissue. When this happens, the most common symptoms include abnormal vaginal bleeding, such as bleeding after sex, bleeding after menopause, bleeding and spotting between periods, and having longer or heavier (menstrual) periods than usual. Bleeding after douching, or after a pelvic exam is a common symptom of cervical cancer but not pre-cancer.

Some of the women have an unusual discharge from the vagina. This discharge may contain some blood and may occur between menstrual cycles or after menopause. Pain during sex occurs among some women during the early cancerous and pre-cancerous state.

Women with clinically invasive cancer usually have symptoms and signs such as post coital bleeding, recurrent cystitis, and exophytic and cervical lesions. In the clinical worst state of the disease, the pelvic lymph nodes are invaded and the original lesion infiltrates the parametrium and obstructs the urethra, which can cause renal failure and uremia. Pressure against nerve trunks and the sacral plexus produces persistent pain.

2.5 Genital Warts

Genital warts caused by either HPV 6 or 11 have similar clinical manifestations or histology. Several studies have indicated that about 100% of genital warts are caused by HPV 6 and 11. Genital warts do not usually cause serious morbidity and mortality but cause psychological morbidity. Even though genital warts persist for a very long time, they rarely progress to cancer (Lacey et al, 2006).

Genital warts are highly infectious, with a transmission rate of about 65% within sexual partnerships from an infected to a susceptible sexual partner, and an incubation period of

between three weeks and eight months, with the majority developing warts at around two to three months. Once genital warts have developed, they may show minimal change over time, become more numerous or larger, or regress spontaneously (Lacey et al, 2006).

Studies have shown that infected persons have been able to clear the genital warts within a short time. About 5% to 20% of infected persons have been able to clear the genital warts without active treatment (Lacey et al, 2006).

Regressing genital warts contain significantly moreCD4positive T cells, both within the stroma underlying the lesions and the condylomata themselves, and greater expression of activation markers. There is no report of the rate of spontaneous regression that may occur in the longer term (Lacey et al, 2006).

Although it is possible for 90% of HPV infections to be cleared by the body within two years of infection, cells may undergo a latency period, with the first occurrence or a recurrence of symptoms happening months or years later (Juckett et al, 2010).

Following genital warts clearance with therapy using active interventions, recurrence is common and is often seen within three months in 25% of cases, although rates of up to 67% have been observed. Recurrences are observed to often occur at sites of previous lesions and in these cases HPV infection in stem cells or slow-turnover cells at the site of previous clearance has persisted and then reactivated. The proportion of HPV-6and HPV-11 infections that are either completely cleared or persist in a latent form after clinical resolution is unknown, and, indeed, animal models suggest that both outcomes can occur (Lacey et al, 2006). HPV 6 and 11 penetrate epithelial cells through microscopic abrasions in the genital area, which occur during sexual activity.

2.6 Symptoms and Signs of Genital Warts

Most HPV infections have no signs or symptoms. People can be infected with HPV 6 or 11 and pass the infection on to another person without knowing. Thus, People often do not have any symptoms from genital warts. However, some people do get visible genital warts. The genital warts usually do not hurt or itch, which is one reason why people may not know they have them.

Genital warts appear in various sizes and shapes. Some people get a few warts. Others get many warts. The most common signs of these warts include small, scattered bumps that are skin-colored or a bit darker, a cluster of bumps that look like cauliflower and growths in the genital area that can be raised or flat and smooth or rough. Sometimes, the warts itch, burn, hurt, or bleed in some patients.

Genital warts often occur in clusters and can be microscopic or can spread into large masses in the genital area. In other cases they look like small stalks. In women they can occur on the outside and inside of the vagina, cervix, the uterus and the anus.

Studies have shown that when the genital warts are present, the virus may be more contagious. However, infected persons with HPV 6 and 11 can still pass the infection to another person even though the genital warts may not be visible.

2.7 Risk Factors

Sexual contact with an infected partner is essential in the epidemiological chain of HPV transmission. The risk factors of HPV infections are sexual activity markers (high number of sexual partners, young age at sexual debut, and recent new sexual partner), other risk factors of HPV infection include young age, co-infections, long-term oral contraceptive use,

smoking, immune suppression, and multi parity. However, certain genetic polymorphisms in the human leukocyte antigen system and nutrition are possibly associated with reduced risk of HPV infection (Trottier & Franco, 2006).

Knowledge of risk factors especially sexual behaviour and sexual networking in populations is important in the understanding of HPV transmission. Many sexual behaviours increase the risk of getting HPV (Burchell et al, 2006).

2.7.1 Sexual Debut

Age at sexual debut may increase risk of HPV infection either as a marker for other sexual behaviours (e.g. young age at sexual debut may be associated with a greater lifetime number of sexual partners or with propensity to engage new sexual partners more frequently), or as a true causal risk factor due to greater cervical ectopy during adolescence (Trottier & Franco, 2006).

Biological mechanisms, including cervical immaturity, inadequate production of protective cervical mucus and increased in cervical ectopy, may make younger women and adolescents more susceptible to HPV infection.(Burchell et al, 2006). This could account for higher prevalence rate of HPV infection among young women.

Several studies have shown that early sexual debut is a risk factor for HPV infection. The relationship between sexual debut and HPV infection is not clear but many studies have suggested that with earlier sexual debut there is the tendency to have many sexual partners in your life time. The incidence of early sexual debut is decreasing in developed countries while increasing in Sub Saharan Africa with increasing extra marital affairs (Burchell et al, 2006).

2.7.2 Number of Partners

The associations between numbers of new and recent sexual partners and the possibility of detecting HPV-DNA from the female genital specimen are strong and consistent. Also having different sexual partners in your sexual life time increases the risk of being infected with

HPV since the rate of acquisition of partners play a significant role in the transmission of sexually remitted infections (Burchell et al, 2006).

The sexual behaviours of male partners are critical for female acquisition of HPV infection. Female HPV prevalence and acquisition have been positively associated with women's estimates of their male partners' lifetime number of partners or not knowing a male partner's prior sexual history (Burchell et al, 2006).

Patterns of sexual networking are also critical for HPV transmission dynamics. Sexual networks are about individuals who are sexually connected, either directly or indirectly. The network features that increase HPV transmission are larger network size, higher sexual contacts and the nature of sexual mixing (Burchell et al, 2006).

The timing of sexual partnerships plays a role in determining the spread and acquisition of HPV. Studies have shown that frequent change of partners and having sex with new partners within a short time increases the risk of acquisition of HPV. Knowing a partner for more than eight months has been associated with a lower risk of HPV acquisition among women (Burchell et al, 2006).

2.7.3 Other Factors

Apart from sexual activity markers, ie, high number of sexual partners, young age at sexual debut, and recent new sexual partner, other risk factors of HPV infection include young age, coinfections, long-term oral contraceptive use, smoking, immunosuppression, and multiparity. Several studies have established associations between these factors and acquisition of HPV infection (Trottier & Franco, 2006).

Age, immune status, marital status, smoking, literacy, income, housing, parity, partner with several partners are all seen as factors that increase the risk of getting HPV infections. The above mention factors increase one's risk of acquiring sexually transmitted infections. There

are also some genetic factors which predispose some women to the acquiring of HPV and subsequently developing cervical cancer (Trottier & Franco, 2006).

Other factors such as infection with other sexually transmittable infections may increase the susceptibility of HPV infection by cervical inflammation or microabrasions, or facilitate persistence of HPV infection through immunological mechanisms. The similar sexual behaviour risk factor profiles for HPV and other sexually transmitted infections , however, make it difficult to discern whether other sexually transmitted infections are simply markers for exposure to HPV or act as a true cofactors by increasing susceptibility or infectivity(Burchell et al, 2006).

2.8 Prevention of HPV, Cervical Cancer and Genital Warts

The best way to prevent getting an HPV infection is to avoid direct contact with the virus, which is transmitted by skin-to-skin contact. It is difficult to avoid skin-to-contact with our love ones in our relationships. Also, in most cases of HPV infection, there are not visible signs for us to know in order to avoid direct skin contact with infected individuals. So the way forward is either primary or secondary prevention of HPV infection.

Cervical cancer is a malignant neoplastic disease for which public health prevention initiatives have had the greatest success. Public health prevention initiatives have been able to reduce the morbidity and mortality of HPV and cervical cancers. Well organized screening using Pap smear has been able to reduce the cervical cancer burden by 75% in the high income countries during the past five decades. An estimated 40 million to 50 million Pap smears are done annually in United States to detect cancer and the precursor cells (Trottier & Franco, 2006).

It is observed that about 60% of cervical cancer cases occur in women who have not Pap smear test for the last three years. Therefore regular screening, good sexual behaviour and good life styles can help prevent HPV infection and cervical cancer (Trottier & Franco,

2006). Intensive public health education on good sexual behaviours has shown to be effective in reducing the acquisition of HPV infection. Vaccination against HPV is an important primary method of preventing the transmission of HPV infections.

2.9 HPV Vaccination

HPV vaccination has the potential to significantly decrease the incidence of HPV type-specific cervical cancers and the burden associated with such infections. High vaccine coverage, sustained over many decades, with a long duration of vaccine-conferred protection would have a great impact on cervical cancer incidence. Even with high uptake of the vaccine, however, a statistically noticeable reduction of the burden of cervical cancer via HPV vaccination is unlikely to be observed for at least a decade or longer because of the latency required for averted high-grade lesions to progress to invasive disease (Trottier & Franco, 2006).

HPV vaccines are given as a series of three shots over 6 months to protect against HPV infection and the health problems that HPV infection can cause. Two vaccines (Cervarix and Gardasil) protect against cervical cancers in women. One vaccine (Gardasil) also protects against genital warts and cancers of the anus, vagina and vulva. Both vaccines are available for females. Only Gardasil is available for males (CDC, 2013).

HPV vaccines offer the best protection to girls and boys who receive all three vaccine doses and have time to develop an immune response before being sexually active with another person. That's why HPV vaccination is recommended for preteen girls and boys at age 11 or 12 years (CDC, 2013).

Recent studies on the safety and efficacy of vaccines against HPV has shown almost 100% efficacies in preventing persistent infections and development of cervical lesions. Most of these vaccines are against HPV 16 and 18 which cause about 75% of all cervical cancers. The vaccines have the potential of reducing HPV 16 and 18 (Trottier & Franco, 2006).

2.10 HPV Screening

2.10.1 Pap Cytology

It is generally agreed that cytology screening for cancer of the cervix has been effective in reducing the incidence and mortality from the disease in many developed countries. It is the organised programmes that have shown the greatest effect, while using fewer resources than the unorganised programmes. Pap smear or Pap cytology is most common cytological tests used for cervical cancer screening.

Pap cytology is the most widely used screening test for cervical cancer screening. Pap smear test has been successful for the past decades in reducing the cervical cancer burden worldwide. With all the successes it has limitations and challenges especially in developing countries. It is based on interpretation of morphological alterations in cervical smear which is highly subjective (Trottier & Franco, 2006).

The way the samples are collected influences the result; therefore, sampling must be taken properly. Errors in the interpretation of the smears can occur as a result of tiredness as the examiners often examine so many slides. Recent studies have indicated that in detecting CIN, Pap cytology has 51% sensitivity and 98 % specificity. This means that Pap cytology has a high false-negative rate (Trottier & Franco, 2006).

The advent of liquid-based cytology has helped to mitigate the problem of efficiency in processing smears in screening programs, but the limitations of cytology remain the same. This low sensitivity for an individual testing opportunity has to be compensated by the requirement to have women entering screening age with an initially negative smear to repeat their tests at least twice over the next 2 to 3 years before they can be safely followed as part of a routine screening schedule (Trottier & Franco, 2006).

2.10.2 Visual inspection with acetic acid (VIA)

This involves non-magnified visualization of uterine cervix soaked 3-5% acetic acid. Financial and technical challenges in Pap smear test in developing countries led to examination by VIA. It is a basic test for screening purpose. Some studies have indicated that the sensitivity and specificity of VIA are 84% and 82% respectively in detecting high grade precancerous lesions. While Pap cytology reporting is only done by pathologist, VIA reporting is done by several trained personnel ranging from doctors to non-medical personnel (WHO, 2002).

In most of the studies where cytology and VIA have been provided under the same conditions, the sensitivity of VIA was found to be similar to that of cytology, whereas its specificity was consistently lower. A wide range of personnel ranging from doctors, nurses and other allied health workers to non-medical personnel has been involved in the administration and reporting of VIA results (WHO, 2002).

The major limitations of VIA include: low specificity (generally less than 85%), which can lead to over-investigation and over-treatment of screen positive women and lack of standardized methods of quality control, training and competency evaluation. Furthermore, it is limited in its ability to detect endocervical disease. The major strengths of VIA include its simplicity and low cost, real time availability of results and potential for immediate linkage with investigations/treatment, consistent estimates of accuracy, feasibility to be offered in low resource settings and the possibility of rapid training of providers (WHO, 2002).

2.10.3 Molecular Based Technologies

Molecular based technologies involve the use of techniques to detect the HPV- DNA in cervical samples. Of the molecular-based technologies for cervical cancer screening, HPV testing is eliciting the greatest interest in Western countries. There are primarily two technologies for this purpose. The Hybrid Capture aassay is currently the most widely used in clinical and screening settings. The Hybrid Capture is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection in cervical specimens of HPV DNA of 13 HR genotypes, defined as those HPV genotypes that are associated with cervical cancer: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 (Trottier & Franco, 2006).

Recently, it is playing a major role in the reduction of HPV and cervical cancer burden in the developed countries. There are basically two technologies adopted by these countries for the screening purposes. These technologies are Hybrid Capture assay and PCR. In order words, molecular based technologies involve the use of polymerase chain reaction (PCR) to detect the DNA of HPV in cervical specimens. Some Meta –analysis done indicated that the sensitivity of PCR is about 30% more than Pap smear and specificity 10% less than Pap smear test. Others indicated that the sensitivity of PCR is about 100% (Trottier & Franco, 2006).

The advantages of HPV-DNA tests as compared to cytology are:

• The objectivity of the test resulting in very low inter- and intra-observer variability.

• The possibility of almost complete automation of the process. This should ensure high throughput at a standard level of quality.

• Built-in quality control procedures.

• Opportunities for self-sampling for HPV DNA in some populations with limitations in health care facilities and manpower, albeit with some loss of sensitivity.

• The high sensitivity of the HPV DNA test to identify HSIL in women aged 30 and above.

• Gains in effectiveness could be achieved by increasing the length of the interval between screens and reducing the total number of lifetime screens required (WHO, 2002).

CHAPTER THREE

METHODOLOGY

3.1 Methods

3.2 Study Location

The study was conducted at the Family Planning and HPV Screening Centre (FPHC) of Ridge Hospital in Accra. Ridge Hospital is located in north ridge in Accra, the capital of Ghana. Ridge Hospital is the regional hospital of Greater Accra region. North ridge is a Sub-Metropolis in the Accra Metropolitan Assembly, bounded to the south by the Castle Road, Kanda to the west and to the east by the Independence Avenue. The hospital is made up of several departments including surgical, medical, obstetrics and gynaecology, paediatrics and FPHC. The FPHC offers services in family planning, gynaecological, outreach, health education, counselling, HPV and breast cancer screening.

The Family Planning and HPV screening centre (FPHC) is an integral part of the gynaecological department of the hospital. There are two specialist gynaecologists in the unit with five public health nurses and three midwives.

Most patients visiting the FPHC were usually referred for the cervical cancer screening. Cervical samples for investigations were taken by the gynaecologists and midwives. Study participants were recruited from the patients who visited the cervical screening centre. Consent was obtained from them before they were enrolled into the study.

3.3 Study Design

This was a quantitative cross- sectional study on the prevalence of HPV infection among women aged 19 to 60 years, attending the Family Planning and HPV Screening Centre

(FPHC) of Ridge Hospital from May to June, 2013. Women who visited the centre for cervical cancer screening and consented to participate were enrolled into the study. Questionnaires were administered to the participants to collect data on their socio demographic characteristics, sexual, gynaecological and medical history. Cervical samples were taken for the detection of HPV 6, 11, 16 and 18 using polymerase chain reaction (PCR). The presence or absence of HPV was the dependant or outcome variable whiles the sociodemographic characteristics, sexual, gynaecological and medical history were the independent variables.

3.4 Study Variables

The dependent or the outcome variable was the presence or absence of HPV 6, 11, 16 and 18.

The independent variables were sociodemographic characteristics, gynaecological, medical and sexual history.

3.5 Study population

Women aged 19 to 60 years attending the Family Planning and HPV Screening Centre of the Ridge Hospital between May and June 2013.

3.6 Inclusion Criteria

Women:

1. Aged 19 to 60 years
2. undergoing HPV screening at FPHC
3. Who consented to participate in the study

Exclusion Criteria

Women who:

1. were pregnant

2. had undergone hysterectomy
3. had been treated for HPVs
4. had vaginal bleeding or discharge

3.7 Sample size

The FPHC of Ridge Hospital received 350 to 500 women per month of which 150 to 200 came for cervical screening. Using 21.5 % prevalence of HPV among women in West Africa (WHO, 2009) a minimum of 260 sample size was calculated. A maximum of 286 was proposed to be collected in the study to take care of 10% (26) non-response. The sample size was calculated using the formula below at 95 % confidence interval.

$$\mathbf{n} = \frac{\mathbf{t}^2 \times \mathbf{p(1-p)}}{\mathbf{m}^2}$$

Description:

n = required sample size

t = confidence level at 95% (standard value of 1.96)

p = estimated prevalence of HPV in West Africa

m = margin of error at 5% (standard value of 0.05)

3.8 Data Collection Tool

A structured questionnaire was used to elicit information on the sociodemographic characteristics, gynaecological, medical and sexual history of each participant.

3.9 Sample Collection

Following completion of the questionnaire by the principal investigator, a gynaecologist took ectocervical and endocervical samples and from each participant for the extraction of DNA for the detection of HPV. Briefly, a sterile vaginal speculum was inserted into the vagina of

the participant and using a sterile Ayre's spatula, exfoliated cells were obtained from the transformation zone of the cervix. Endocervical brush was also used to take endocervical samples. The spatula was then rinsed and the brush was cut in a sterile phosphate buffer (PBS, PH 7.4) to collect the tissues. This phosphate buffer also served as a transport medium for the samples to be carried to the laboratory for analysis.

3.10 DNA Extraction

DNA was extracted using Fermentas life sciences viral extraction kit. It was done according to the extraction protocol of the kit. Briefly, 400µl of each sample was taken into eppindorf tubes and span at 200 rpm for five minutes. 200 µl of the concentrate was pipetted into a different eppindorf tube, 200 µl of lyses buffer and 50 µl of proteinase k were added, vortexed and incubated at 55 ^{o}C for 30 minutes. This mixture was transferred into silicon columns for purification and elusion (isolation) of the DNA. The DNA was stored at -20 ^{o}C to be used later for amplification.

3.11 Quantification, Purity and Integrity of DNA

Extracted DNA was quantified, checked for purity and integrity using ThemoScientific Nanodrop 2000. DNA purity of 1.5 to 2.5 at 260nm /280nm was accepted for amplification.

3.12 PCR Primers and Amplification

HPV dual priming oligonucleotide (DPO) primers from Seegene, which was a multiplex system for HPV genotypes, was used for amplification. The amplification was done according to the protocol below:

The PCR master mix was prepared by measuring 4 µl of 5x HPV primer mix, 3 µl of 8-Mop solution, 10 µl of 2x multiplex master mix in to the PCR tubes for tests and controls. The mixture was mixed by votexing and centrifuged briefly. 3 µl of extracted DNA from the samples, positive control or negative control were added to the PCR mix. This was vortexed and span down in the PCR tubes using the micro centrifuge for 60 seconds. The PCR tubes

containing the mixture were placed in the thermocycler (PCR equipment) which is preheated for amplification. The amplification was done at 40 cycles starting from 94 ^{o}C for 15minutes, followed by the 40 cycles at (94 ^{o}C for 0.5 minutes, 60 ^{o}C for 1.5minutes, and 72 ^{o}C for 1.5minutes).

3.13 PCR Product Visualization

Amplified DNA fragments were separated in 2% agarose gel by electrophoresis stained with 10% cyber green. The UV-illuminated gels were viewed, photographed and documented using Wealtec Imaging System. Electrophoresis was read by comparison with the DNA molecular weight ladder/ markers, and a positive result identified by the corresponding base pairs (bp) on the ladder/markers. HPV 16 corresponded with 588 bp, HPV 6/11 corresponded with 302bp, and whiles HPV 18 corresponded with 230bp on the ladder/markers.

3.14 Disposal of Samples and Amplicons

The leftover samples, the amplicons and the waste generated from the process in the closed waste containers, were sterilized at 121 degree Celsius for 20 minutes and finally incinerated at the Korle-Bu Teaching Hospital.

3.15 Data Analysis

A data analysis was done using Stata 11. The ages were grouped into three categories 19 to 30, 31 to 40, 41 to 60 years. Educational levels were classified as: no formal education, primary education, secondary education and tertiary education. Age at first sexual intercourse was grouped into less than or equal to 18 years and greater than 18 years. Number of life time partners was classified as 1, 2 to 3 and 3+. The frequencies for each of the variables above were calculated. The means, maximum and minimum values for age, age at first sexual intercourse and number of lifetime sexual partners, number of pregnancies and parity were determined. Odds ratios for HPV positivity and corresponding 95% confidence intervals was calculated by means of logistic regression equations.

3.16 Quality Control

The laboratory work was done at the molecular laboratory of School of Allied Health Sciences, College of Health Sciences of the University of Ghana. This was done according to the standard protocol of good laboratory practice. All quality control procedures were adhered to. There was an in-built quality control substance, 8-methoxypsoralen, to take care of contaminations and failure to follow protocol. All the reagents, consumables, instruments and equipment were all CE and FDA marked.

Administered questionnaires were reviewed to safeguard the consistency and quality of the data. Risk of multiple entries was minimized by coding the questionnaires before the data entry. Collected data was safeguarded for the sake of the privacy of respondents.

3.17 Pretesting of Questionnaires

The questionnaires were pretested at the Trust Hospital Gynaecology OPD, a public hospital within the same locality. The necessary corrections were made before the final use of the questionnaire.

3.18 Ethical Consideration

The protocol for the study was submitted to the ethical committee of the Ghana Health Service for approval.

3.19 Consent form

Subjects who were included in the study signed a consent form to take part in the study. The consent was read and interpreted to the understanding of a participant who could not read nor understood English. The participants were allowed to withdraw from the study at any time without any undesirable consequences.

3.20 Approval from the Study Area

Consent was sought from the FPHC of the Ridge Hospital as well as the molecular laboratory of the School of Allied Health Sciences, College of Health Sciences of the University of Ghana (That was where the laboratory work was done).

3.21 Possible benefits

Respondents were informed that taking part in the study might not bring them immediate or direct benefits, however, their participation would help determine the prevalence of human papilloma virus among women attending the ridge hospital and the recommendations made might go a long way to influence policy in order to address issues of cervical cancer.

3.22 Possible risk/discomfort

Respondents were informed that this study had no known risk but answering these questions might be boring to them or might pry into their personal life. They were allowed to withdraw from the interview anytime they wish. They were also allowed to skip answering some questions that they might find uncomfortable, even though answers to such questions were handled confidentially.

3.23 Confidentiality

All study materials (questionnaires, informed consent form) were stored in a locked file cabinet in the office of the principal investigator. With the exception of the research team and the ethics committee, access to the data they provided us was strictly protected.

3.24 Compensation

Eligible persons who consented to participate in the study were not given any monetary compensation.

CHAPTER FOUR

RESULTS

4.1 Sociodemographic Characteristics of Respondents

A total of 201 women were sampled, interviewed and cervical specimens were taken at Ridge Hospital, Accra to determine the prevalence of human papillomavirus and the associated risk factors. A minimum of 260 and a maximum of 286 women were proposed as the research sample size which was not obtained. However this shortfall in sample size did not affect the results as the overall prevalence of HPV (15.92%) at 95% confident interval was within the range of (11.15 - 21.72). Only the women who came for cervical cancer screening were invited to participate in the study in order to have a uniform set of women for the study. All the women who came for cervical cancer screening in that mouth agreed to take part in the study without a single refusal. Therefore, 201 women came for the screening and they were all enrolled in to the study. These results can only be associated with the women who visit the Ridge Hospital and to a large extend women in Greater Accra Region since the hospital is the regional hospital. All of the women who participated in the study live in Accra and of cause will not be different from other women living in Greater Accra Region.

The ages of the participants who were enrolled in to the study ranged from 19 to 60 years and the mean age was 38.43 ± 11.81 years. The majority (89.55%) had at least primary level of education with 10.45% having no formal education. One hundred and sixty one (80.1%) of the women were employed and forty (19.9%) were unemployed. A total of sixty four (31.84 %) of the women earned monthly income below GH¢ 100 whiles thirty four (14.715) earned more than GH¢ 1000. Fifteen (7.46%) of the participants had no permanent place of residence. One hundred and thirty three (66.17%) lived most of their lives in the city and ten (4.98) lived in the village. The majority (86.07%) of the participants were Christians and

13.93% Muslims. Sixty three (31. 34%) of the women were Akan, thirty two (15.92%) Ewe, seventy one (35.32%) Ga and thirty five (17.37%) belong to other ethnic groups. (Table1).

Table 2 shows the output of logistic regression model constructed to determine strength of association between demographic factors and the prevalence of HPV infection at 5% significant level. The results of the tests showed that there was no statistically significant association between any of the sociodemographic variables , that is, age (OR=1.35, p=0.20), educational background (OR=1.03, p=0.86), occupation (OR=1.14, p=0.36), income level (OR=0.86, p=0.42), housing (OR=0.79, p=0.43), permanent place of residence (OR= 1.18, p=0.60) , religion (OR=1.68, p=0.42) , ethnicity (OR=0.83, P=0.15) and the prevalence of human papillomavirus infection .

Table 1: Sociodemographic Characteristics and the Prevalence of Human Papillomavirus

Socio-demographic Characteristics	N=201	n= 32	Prevalence of HPV (%)	Chi Squire	P-Value
Age (years)					
19-30	64	8	12.50		
31-40	58	8	13.79	1.86	0.39
41-60	79	16	20.25		
	201	**32**	**46.54**		
Educational background					
No formal	21	4	19.05		
Primary	12	2	16.67		
JHS/Middle	38	4	10.53	1.19	0.88
Secondary	69	11	15.94		
tertiary	61	11	18.03		
	201	**32**	**80.22**		
Occupation					
Unemployed	40	4	10.00		
Housewife	13	3	23.08		
Manual/menial	8	2	25.00	3.12	0.54
Self employed	92	13	14.13		
Civil servant	48	10	20.83		
	201	**32**	**93.04**		
Income(GH¢)					
< 100	64	12	18.75		
100-500	73	12	16.44	1.22	0.75
600-1000	30	3	10.00		
>1000	34	5	14.71		
	201	**32**	**59.90**		
Housing					
No permanent Residence	15	3	20.00		
Self-contained house	73	13	17.81	0.64	0.73
Compound house	113	16	14.16		
	201	**32**	**51.97**		
Permanent place of residence					
City	133	18	13.53		
Town	58	14	24.14	5.39	0.07
vilage	10	0	0.00		
	201	**32**	**37.67**		
Religion					
Islam	28	3	10.71	0.66	0.42
Christianity	173	29	16.76		
	201	**32**	**27.47**		
Ethnicity					
Akan	63	13	20.63		
Dagbani	12	3	25.00		
Ewe	32	3	9.38	3.78	0.58
Ga	71	11	15.49		
House	9	1	11.11		
Others	14	1	7.14		
	201	**32**	**88.75**		

N= total number of participants
n=total number of positive HPV-DNA

Table 2 : Odd Ratios of Sociodemographic Characteristics and Prevalence of Human Papillomavirus Using Logistic Regression

Socio-demographic Characteristics	Odd Ratio	95%CI	P-Value
Age (years)	1.35	0.85-2.15	0.20
19-30	1.00		
31-40	1.12	0.39-3.21	0.83
41-60	1.78	0.71-4.47	0.22
Educational background	1.03	0.76-1.40	0.86
No formal	1.00		
Primary	0.85	0.13-5.51	0.87
JHS/Middle	0.50	0.11-2.25	0.37
Secondary	0.81	0.23-2.86	0.74
Tertiary	0.93	0.26-3.33	0.92
Occupation	1.14	0.86-1.51	0.36
Unemployed	1.00		
Housewife	2.70	0.52-14.09	0.24
Manual/menial	3.00	0.45-20.15	0.26
Self employed	1.48	0.45-4.86	0.52
Civil servant	2.37	0.68-8.23	0.18
Income(GH¢)	0.86	0.35-1.24	0.42
< 100	1.00		
100-500	0.85	0.35-2.06	0.72
600-1000	0.48	0.13-1.85	0.29
>1000	0.75	0.24-2.33	0.62
Housing	0.79	0.44-1.41	0.43
No permanent Residence	1.00		
Self-contained house	0.87	0.21-3.51	0.84
Compound house	0.66	0.18-2.60	0.55
Permanent place of residence	1.18	0.63-2.22	0.60
City	1.00		
Town	2.03	0.93-4.43	0.08
Village	0.00		
Religion	1.68	0.47-5.92	0.42
Islam	1.00		
Christianity	1.68	0.47-5.92	0.42
Ethnicity	0.83	0.65-1.07	0.15
Akan	1.00		
Dagbani	1.28	0.30-5.42	0.74
Ewe	0.40	0.10-1.51	0.18
Ga	0.75	0.29-1.71	0.44
House	0.48	0.06-4.19	0.51
Others	0.30	0.04-2.47	0.26

Table 3: Sexual History and the Prevalence of Human Papillomavirus

Sexual History	N=201	n=32	Prevalence of HPV (%)	Chi Squire	P-Value
Marital status					
Divorced/separated/widowed	19	1	5.26		
Married/cohabited	120	18	15.00	2.87	0.24
Single	62	13	20.97		
	201	**32**	**41.23**		
Age at sexual debut(years)					
>18	77	9	11.69	1.67	0.20
<18	124	23	18.55		
	201	**32**	**30.24**		
Multiple partners					
1	149	18	12.08	8.30	0.04
2 or more	52	14	26.92		
	201	**32**	**39.00**		
Partners extra sexual relationships					
No extra partners	108	19	17.59	0.49	0.49
Extra partners	93	13	13.98		
	201	**32**	**31.57**		
Acquisition of new partners					
No partner before current partner	53	7	13.21	0.40	0.53
Had partner before current partner	148	25	16.89		
	201	**32**	**30.10**		
No partner after current partner	110	20	18.18	0.93	0.34
Have partner after current partner	91	12	13.19		
	201	**32**	**31.37**		
Life time Condom use					
Does not use condom	143	22	15.38	0.11	0.74
Uses condom	58	10	17.24		
	201	**32**	**31.57**		

N= total number of participants
n= total number of positive HPV-DNA

Table 4: Odd Ratios of Sexual History and Prevalence of Human Papillomavirus Using Logistic Regression

Sexual History	Odd Ratio	95%CI	P-Value
Marital status	1.74	0.90-3.34	0.10
Divorced/separated/widowed	1.00		
Married/cohabited	3.18	0.40-25.3	0.28
Single	4.78	0.58-39.1	0.15
Age at sexual debut(years)	1.72	0.75-3.94	0.20
>18	1.00		
<18	1.72	0.75-3.94	0.20
Multiple partners	2.68	1.22-5.86	0.014
1	1.00		
2 or more	2.68	1.22-5.86	0.014
Partners extra sexual relationships	0.76	0.35-1.63	0.49
No extra partners	1.00		
Extra partners	0.76	0.35-1.63	0.49
Acquisition of new partners			
Before	1.34	0.54-3.30	0.53
No partner before current partner	1.00		
Had partner before current partner	1.34	0.54-3.30	0.53
After	0.68	0.31-1.49	0.34
No partner after current partner	1.00		
Have partner after current partner	0.68	0.31-1.49	0.34
Life time Condom use	1.15	0.51-2.60	0.75
Does not use condom	1.00		
Uses condom	1.15	0.51-2.60	0.75

4.2 Sexual History of the Participants

A total of 201 participants were interviewed to collect data on their sexual history. Sixty two (38.85%) of the women were single, one hundred and twenty (59.70%) married or cohabiting and nineteen (9.45%) divorced, separated or widowed. A hundred and twenty four (61.69%) of them had sex before age 18. The age at sexual debut ranged from 12 to 30 years with a mean age of 18.7 ± 3.46 years. Fifty two (25.87%) of the participants had multiple partners and one hundred and forty nine (74.13%) had one partner. The number of partners ranged from 1 to 4 and the mean for sexual partners was 1.38 ± 0.72. Ninety three (46.27%) of the women lived in a relationship where their partners had extra sexual relationships whiles one hundred and eight (53.73%) of them lived in relationships without extra sexual partners. Majority (73.63%) had partners before meeting the current partner whiles ninety one (45.27%) especially the singles had new partners in addition to the current partner after meeting the current partner. The majority (71.14%) of the women had partners who never used condom whiles (28.86%) of the women had partners who ever used condom (Table 3).

Results in Table 4 show the logistic regression module constructed to determine strength of association between sexual history of the participants and the prevalence of human paillomavirus infection at 5% significant level. The results show that there was no statistically significant association between any of these variables, thus, marital status (OR=1.74, p=0.1), age at sexual debut (OR=1.72, p=0.20), partners extra sexual relationship (OR=0.76, p=0.49), acquisition of new partners before the current partner (OR=1.34, p= 0.53), acquisition of additional partners after current partner (OR=0.68, p=0.34), condom used by partner (OR=1.15, p=0.75) and the prevalence of human papillomavirus infection. A statistically significant association was however observed between having multiple partners (OR=2.68, p=0.014) and the prevalence of human papillomavirus. The association showed that having multiple partners increased the odd of HPV infection about 3 folds.

4.3 Gynaecological History of the Participants

From Table 5, a total number of 93 (46.27%) of the study participants ever used modern contraception. The number of pregnancies ranged from 0 to 9 and the mean pregnancies was 2.40 ± 2.09. Majority (79.10%) of the study participants had ever been pregnant and 20.90% had never been pregnant. Parity ranged from 0 to 9 children and the mean was 1.68 ± 1.74. Majority (64.68%) of the women had one child whiles 35.32% never had one child.

Table 5 : Gynaecological history and the Prevalence of human papillomavirus

Gynaecological History	N=201	n=32	Prevalence of HPV (%)	Chi Squire	P-Value
Modern Contraception use					
Never used contraception	108	18	16.67	0.10	0.76
Ever used contraception	93	14	15.05		
	201	**32**	**31.72**		
Number of pregnancies					
0	42	10	23.81		
1-3	111	15	13.51	2.50	0.29
>3	48	7	14.58		
	201	**32**	**51.90**		
Parity					
0	71	15	21.13		
1-3	102	12	11.76	2.83	0.24
>3	28	5	17.86		
	201	**32**	**50.75**		

N= total number of participants
n= total number of positive HPV-DNA

Table 6 shows the results of logistic regression model constructed to determine the association between gynaecological history of the participants and the prevalence of human papillomavirus infection at 5% significant level. The results show that there was no

statistically significant association between any of the variables of gynaecological history, thus, modern contraception use (OR=0.89, p=0.76), pregnancies (OR=0.72, p=0.76), parity (OR=0.76, p=0.36) and the prevalence of human papillomavirus infection.

Table 6 : Odd ratios of gynaecological history and prevalence of human papillomavirus using logistic regression

Gynaecological History	Odd Ratio	95%CI	P-Value
Modern Contraception use	0.89	0.41-1.90	0.76
Never used contraception	1.00		
Ever used contraception	0.89	0.41-1.90	0.76
Number of pregnancies	0.72	0.41-1.27	0.26
0	1.00		
1-3	0.50	0.20-1.22	0.13
>3	0.55	0.19-1.59	0.27
Parity	0.76	0.43-1.36	0.36
0	1.00		
1-3	0.50	0.22-1.14	0.10
>3	0.81	0.26-2.49	0.72

4.4 Medical History of the Participants

The majority 190 (94.53%) of the participants never smoked or had partners who did smoke. One hundred and twenty four (61.69 %) of the women never consumed alcohol while seventy seven (38.31%) ever consumed alcohol. Twenty four (11.94 %) of the participants suffered from hypertension, four (1.99%) from genital warts and sixty three (31.34%) had sexually transmitted infections whiles one hundred and one (54.73%) were not diagnosed of any of the conditions above (Table 7).

Table 8 shows the association between medical history and the prevalence of human papillomavirus infection at 5% significant level. The results show that there was no statistically significant association between any of the variables of medical history, that is, smoking (OR=1.18, p=0.83), alcohol (OR= 1.45, p=0.37), diseases (OR=1.15, p=0.33) and the prevalence of human paillomavirus infection.

Table 7: Medical history and the Prevalence of human papillomavirus

Medical History	N=201	n=32	Prevalence of HPV (%)	Chi Squire	P-Value
Smoking					
Do not smoke	190	30	15.79	0.04	0.83
Smoke	11	2	18.18		
	201	**32**	**33.97**		
Alcohol					
Do not consume alcohol	77	10	12.99	0.80	0.37
Consume alcohol	124	22	17.74		
	201	**32**	**30.73**		
Diseases					
None	110	14	12.73		
Hypertension	24	6	25.00	4.83	0.44
Genital warts	4	0	0.00		
STI	63	12	19.05		
	201	**32**	**56.78**		

N= total number of participants
n= total number of positive HPV-DNA

Table 8 : Odd ratios of medical history and prevalence of human papillomavirus using logistic regression

Medical History	Odd Ratio	95%CI	P-Value
Smoking	1.18	0.24-5.76	0.83
Do not smoke	1.00		
Smoke	1.18	0.24-5.76	0.83
Alcohol	1.45	0.64-3.24	0.37
Do not consume alcohol	1.00		
Consume alcohol	1.45	0.64-3.24	0.37
Diseases	1.15	0.87-1.51	0.33
None	1.00		
Hypertension	2.29	0.76-1.51	0.13
Genital warts	0.00	-	-
STI	1.61	0.69-3.75	0.27

4.5 Knowledge of Cervical Cancer by Participants

From Table 9, the majority 146 (72.64%) of the women were aware of cervical cancer and the availability of cervical cancer screening services. Only a small number 46(22.89%) of the women however knew that only sexually active women were at risk of contracting human papillomavirus. Sixty one (30.35%) of the women ever screened for human papillomavirus and cervical cancer whiles one hundred and forty never screened for human papillomavirus and cervical cancer. Thirty (14.93%) of the women used Papanicolaou's smear, thirty one (15.42%) used VIA but none used PCR (HPV-DNA) for the screening of human papillomavirus and cervical cancer.

Table 10 shows the results of logistic regression model constructed to determine the strength of association between the knowledge of cervical cancer by the participants and the prevalence of human papillomavirus infection at 5% significant level. The results show that there was no statistically significant association between any of the variables of the knowledge of cervical cancer, thus, awareness (OR=1.77, p=0.24), knowledge of risk group (OR=1.26, p=0.42), screening (OR=1.05, p=0.90), method used by participants (OR=1.02, p=0.93) and the prevalence of human papillomavirus infection.

Table 9: Knowledge of cervical cancer and the Prevalence of human papillomavirus

Knowledge of Cervical Cancer	N=201	n=32	Prevalence of HPV (%)	Chi Squire	P-Value
Awareness					
Not aware	55	6	10.91	1.42	0.23
Aware	146	26	17.81		
	201	**32**	**28.72**		
Risk group					
Do not know	45	6	13.33		
All women	110	17	15.45	0.70	0.71
Women who have had sex	46	9	19.57		
	201	**32**	**48.35**		
Screening					
Never screened	140	22	15.71	0.01	0.90
Ever screened	61	10	16.39		
	201	**32**	**32.10**		
Method used					
Not screened	140	22	15.71		
Pap smear	30	5	16.67	0.02	0.90
VIA	31	5	16.13		
PCR	0	0	0.00		
	201	**32**	**48.51**		

N= total number of participants
n= total number of positive HPV-DNA

Table 10 : Odd ratios of knowledge of cervical cancer and prevalence of human papillomavirus using logistic regression

Knowledge of Cervical Cancer	Odd Ratio	95%CI	P-Value
Awareness	1.77	0.69-4.57	0.24
Not aware	1.00		
Aware	1.77	0.69-4.57	0.24
Risk group	1.26	0.72-2.22	0.42
Do not know	1.00		
All women	1.19	0.44-3.24	0.74
Women who have had sex	1.58	0.51-4.88	0.43
Screening	1.05	0.46-2.37	0.90
Never screened	1.00		
Ever screened	1.05	0.46-1.69	0.93
Method ever used by participants	1.02	0.62-1.69	0.93
Not screened	1.00		
Pap smear	1.07	0.37-3.10	0.90
VIA	1.03	0.36-2.98	0.95
PCR	0	-	-

4.6 Prevalence of Human Papillomavirus among the Women Attending Ridge Hospital

Figure 2 shows the 2% agarose gel electrophoresis photograph of the products of the PCR amplification of the HPV DNA in the cervical samples. The figure shows the results of HPV 6,11,16,18 detection and identification by the corresponding 302, 302, 588 and 320 base pairs on the 2% agarose gel.

The mean age of the women who were HPV DNA positive was 40.16 years (standard deviation, 12.70 years; range, 19 to 60 years), whilst the mean age of the women who were HPV DNA negative was 37.99 years (standard deviation, 11.64years; range, 20 to 60 years). The overall prevalence of HPV 6, 11, 16, 18 or other types (detected collectively) infection among the women was 15.92% (32/201) with 95% confident interval of (11.15 - 21.72). Of the women with HPV 6, 11, 16, 18 or other types detected, 98.51 % (29/32) had one type, 1.49 % (3/32) had two types, and none had three nor four types. Overall prevalence of specific types in the population was 1.00% (2/201) for HPV 6 and 11, 2.99% (6/201) for HPV 16, and 1.99% (4/201) for HPV 18 and 9.95% (20/201) for other HPV types.

Considering the age groups of the participants ,the highest prevalence was seen among women aged 41- 60 years (20.25%) , followed by 13.79% among women age group 31-40 years, and 12.50% among age group 19-30.

With regards to formal education, the prevalence of HPV was 19.05% (highest) among women without formal education and 10.53% (least) among those with JHS or middle school education. The prevalence was seen to be increased among women with manual or menial jobs (25.00%) as compared to 10.00% among the unemployed. With regards to level of income, the infection was highest (18.75%) among the women who earned less than GH¢ 100 and least (10.00%) among those who earned between GH¢ 600-1000. There was an increase of prevalence of HPV (20.00%) among women who had no permanent place of residence compared to those in compound houses (14.16%). It was due to the fact that women who did not have permanent place of residence were more vulnerable than women with permanent residence. The prevalence of HPV was 24.14% among women who lived most of their lives in urban communities with no infection among those who lived in rural communities. With religion, the prevalence was 16.76% among Christian women and 10.71% among Muslim

women. The prevalence of HPV was 25.00% among Dagombas , Akan 20.63, Ga 5.49% and the least was Ewe with 9.38%.

Considering sexual history of the women, the prevalence of HPV was 20.97% among single women followed by the married or cohabited (15.00%) and the least was either divorced, separated or widowed (5.26%). With regards to sexual debut, the highest (18.55%) was among women who had first sex before 18 years as compared to those who had sex after 18 years (11.69%). Human papillomavirus prevalence was 26.92% among those who had multiple partners as compared to those who had one partner (12.08). In acquisition of new partners, the prevalence of HPV was higher (16.89%) among women who had partners before the current partners as compared to those who never had partners before the current partners (13.21%).

The distribution of prevalence of HPV infection associated with gynaecological history was found as follows: the prevalence among women who used modern contraception was 15.05% as compared to those who never used modern contraception (16.67%); the prevalence was highest among those who never became pregnant (23.81%). However, comparing the prevalence of HPV infection between women who had 1-3 pregnancies and women with more than 3 pregnancies, the prevalence was higher among those with more than 3 pregnancies (14.58%) as compared to those with 1-3 pregnancies (13.51%); the same trend of HPV distribution was seen with parity among the women.

The prevalence was 18.18% among women who smoked or had partners who smoked as compared to those who never smoked nor partner ever smoked (15.79%). The prevalence among those who ever consumed alcohol was 17.74% compared to those who never consumed alcohol (12.99%). The distribution of HPV prevalence was 25.00% among the women who were diagnosed of hypertension, followed by those diagnosed of STI (19.05%) and no prevalence (0.00%) among those diagnosed with genital warts. Finally, the prevalence

of HPV was 16.67% among the women who ever screened with Papaniclous smear compared to those screened with VIA (16.13%) with none screened with PCR.

Table 11: Means of the non-categorical risk factors

Risk Factors	Mean	Std. Deviation	Min.	Max.
Age	38.34	11.81	19	60
Age at first sex	18.74	3.46	12	30
Multiple partners	1.38	0.72	1	4
Number of pregnancies	2.40	2.09	0	9
Parity	1.68	1.74	0	9

Figure 2: Gel photograph of Human Papillomavirus PCR products.

CHAPTER FIVE

DISCUSSIONS

The study was to investigate the prevalence of human papillomavirus among women attending Ridge Hospital in Accra. The participants were sampled among women aged 19 to 60 years visiting the family planning and cervical cancer screening centre at Ridge Hospital in Accra. The study participants were stratified into three age groups to determine the prevalence within each age group. It was realised that prevalence of HPV infection increased from 12.50% among age group 19-30 years to 20.25% among those in age group 41-60 years, indicating an increase in prevalence with regards to age. This finding was consistent with a similar study done in Accra with prevalence among age group <30 years (8.8%) and >30years (12.2%) (Domfeh et al., 2008). However , The finding was different from a study done by Keita et al.(2009) in Conakry, Guinea with prevalence among age group <30years (47.2%), age group 35- 44 years (45.3%) and age group 55-64 years (51.4%). The finding from Conakry and findings from Zambia suggest that the prevalence of HPV is in the form of U-shape with higher prevalence at the onset of sex (<30 years), falls around age 40years and peaks at age 50+ years (Ng'andwe et al., 2007).

The women in the study largely had formal education (89.55%), thus, they had education beyond primary school. The family planning and cervical cancer screening centre attracts more formally educated women than women without formal education. With regards to formal education, the finding showed that the prevalence of HPV infection was highest (19.05%) among women without formal education. Though, it was not significant, the finding was consistent with a similar study done in Accra with prevalence among women with formal education being 7.4 % and those without formal education being 57.1% (Domfeh et al.,2008).

Most of the respondents were either self employed or in the civil service. It was realised that the prevalence was lowest among the unemployed (10.00%) but highest among those who do manual or menial jobs (25.00%). The unemployed group was predominantly aged 19- 30 years and that could account for the low level of prevalence among the unemployed. The prevalence was highest among those who do manual or menial jobs because those women are more vulnerable than women in other jobs. The findings were consistent with a study done in Kampala, Uganda where the prevalence was highest (73.4%) among manual workers as compared to other workers (Franceschi et al, 2008).

Majority of the women who were formally educated were employed with a monthly income of more than GH¢ 100. The highest (18.75%) prevalence was among women whose monthly income was below GH¢ 100. Even though the finding was not significant, it was consistent with a cohort study done in India (Thulaseedharan et al., 2012).

A small number (7.46%) of the participants had no permanent place of residence. The prevalence of HPV was higher (20.00%) among this category of women as compared to those with permanent residence (15.59%). It was realised that most of the women who were formally educated had better income and could afford themselves a permanent or better accommodation. The correlation between level of formal education, good income and better accommodation has been established in most studies. One hundred and thirty three (66.17%) of the participants lived in the city and that is obvious because the study centre is located in the city. There was no positive HPV case among those coming from rural communities. This could be due to the small number (10/201) of participants from the rural communities. Comparing those in the urban communities, the prevalence among the women from small cities (24.14%) was higher than those from big cities (13.53%). If where participants lived most of their lives were linked to standard of living, then this finding was consistent with a study done in Kinshasa, DR Congo where prevalence of HPV infection among low standard

of living (18.66%) was higher than that of those with medium standard of living (14.95%) (Sangwa-Lugoma et al., 2011). The majority of the participants were Christians and that could be due to the fact that the predominant religion in Accra is Christianity. The prevalence was higher among Christian women (16.76%) than Muslim women (10.71%). With regards to ethnicity, there were more Gas than the other ethnic groups because the indigenous tribe in Accra is Ga. Despite the fact that the Gas were more than each of the ethnic groups in Accra, Dagombas had most prevalence (25.00%) of HPV infection. It was expected because most Dagomba women are in polygamous marriages.

Several studies have linked sexual history to the prevalence of HPV infection. In this study, about half (59.70%) of the study participants were married or cohabited and 38.85% were single. The prevalence of HPV infection was highest (20.97%) among the women who were single, followed by those who were married with the lowest prevalence being among either divorced, separated or widowed (5.26%). This findings were consistent with a study done in Conakry, Guinea (single=60.5%, married=48.6%, widowed/divorced=47.7%) by Keita et al., (2009). This could be due to the fact that most of the women who were single were in multiple sexual relationships. Majority of the married or cohabited women have ages and similar habits of the singles before marriage or cohabitation. Therefore, they were exposed like the singles before marriage or cohabitation. The infected women might not clear the infection before marriage. Most of the divorced, separated or widowed were older than the other groups and might have had the HPV infection cleared.

More than half of the women had first sex before age 18 years. The earliest age was 12 years and the latest age was 30 years. The prevalence of HPV infection among those who had first sex before age 18 years was higher (18.55%) than those who had first sex after age 18 years (11.69%). This finding was consistent with a study done in Accra where the prevalence was

higher (12.0%) among those who had first sex before age 18 years than those who had first sex after 18 years (8.0%) (Domfeh et al.,2008).

About a quarter (25.87%) of the study participants had multiple sexual partners and the highest number of multiple sexual partners was four. The prevalence of HPV infection was much higher (26.92%) in the group of women with multiple sexual partners than those without multiple sexual partners (12.08%). This finding was significant (p=0.01), thus confirming a strong association between having multiple partners and the prevalence of HPV infection. This association was 2.68 folds among the women with multiple sexual partners as compared to those without multiple sexual partners. These findings were consistent with many studies including studies done in Accra, Ghana by Domfeh et al.(2008) ; Kampala, Uganda by Franceschi et at.(2008); Conakry, Guinea by Keita et al.(2009); Kinshasa, DR Congo by Sangwa-Lugoma et al.(2011). HPV being a sexually transmitted pathogen, like any other sexually transmitted pathogens, having multiple partners increases the risk of being infected with human papillomavirus.

Unexpectedly, about half of the women in the study lived in relationships with their partners having extra sexual relationships. These participants answered yes to the question asking them whether their partners have other sexual partners. The prevalence of HPV infection among the women whose partners had other sexual partners decreased (13.98%) as compared to those whose partners did not have other sexual partners (17.59%). This finding was not significant (p= 0.49) and not expected. Living in a relationship like this is similar to having multiple sexual partners which has the same consequences. Therefore, it was expected that the prevalence should look similar to the prevalence seen in those having multiple sexual partners. This finding was not consistent with the findings in a study done in Conakry, Guinea by Keita et al.(2009) where those living with polygamous husbands had prevalence (52.0%) higher than those without polygamous husbands(47.5%) and another study done in

Kinshasa, DR Congo by Sangwa-Lugoma et al.(2011), although, both findings were not significant (95%CI 0.91 - 1.86).

Majority (73.63%) of the women had partners before meeting the current partner. The prevalence of HPV infection (16.89%) was found to be higher among women who had sexual partners before the current union than those without sexual partners before current union (13.21%). The findings even though was not significant was expected since those who acquired new partners could be more exposed to HPV infection than those who did not acquire new partners. This finding was not consistent with a study done in Kinshasa, DR Congo where it was rather seen that the prevalence decreased in the women who had partners before current union (never acquired new partners OR=1.0, acquired new partner OR=0.69) (Sangwa-Lugoma et al.,2011).

About half (45.27%) of the women had sexual relationships with other partners after meeting the current partners. This finding was seen to be consistent with the percentage of women who had multiple partners. Unexpectedly, there was a reduction in the prevalence of HPV infection (13.19%) among women who had sexual relationship with other partners after current partners as compare to those (18.18%) who did not.

Condom usage was high among 143(71.14%) the partners of the women. Unexpectedly, the prevalence was higher (17.24%) in women who had partners who used condom than those (15.38%) whose partners never used condom. This finding was not significant (p=0.74). Since HPV infection is sexually transmitted infection, it was expected that there should be a reduction in HPV prevalence among the women whose partners ever used condom. This finding was consistent with a study done in Accra, Ghana. Women whose partners ever used condom had prevalence of 12.5% as compared to those whose partners never used condom being 10.4% (Domfeh et al., 2008). This is supported by many studies that showed that condom use cannot protect one against HPV infections. It could be due to the fact that

majority of condom users are occasional users, therefore, they are exposed to infections at the time they are not using condom.

In this study, about halve (46.27%) of the participants were found to have ever use modern contraception. The prevalence of HPV infection among women (15.05%) that ever used modern contraception decreased as compared to those who never used modern contraception (16.67%). This finding was inconsistent with a study done in Conakry, Guinea where the women who ever used hormonal contraception had prevalence of HPV infection (55.6%) higher than those (50.1%) who never used hormonal contraception (Keita et al., 2009).

The majority (79.10%) of the study participants had ever been pregnant with mean pregnancies of 2.40 and the highest number of pregnancies being nine. The highest prevalence of HPV infection was among the women (23.81%) who never became pregnant but prevalence among those who had more than 3 pregnancies (14.58%) was higher than those who had pregnancies 1-3 (13.51%). This finding was consistent with a similar study done in Nigeria by Thomas et al. (2004) in which those who never became pregnant had prevalence of HPV infection of 38.6%, 1-2 pregnancies was 29.1% and more than 3 pregnancies 27.4%. However, the finding was inconsistent with a study done in Kampala, Uganda in which those who never became pregnant had prevalence of HPV infection to be 73.4% compared to those who ever became pregnant - 82.8% (Franceschi et at, 2008).

The mean parity was 1.68 with the highest number of children being nine. The prevalence of HPV infection was highest among the women who never had one child (21.13%) but the prevalence among those who had more than 3 children (17.86%) was higher than those who had 1-3 children (11.76%). This finding was consistent with the studies done in Conakry, Guinea by Keita et al. (2009) and Kinshasa, DR Congo by Sangwa-Lugoma et al. (2011). Despite the fact that literature has found an association between parity and HPV infection, in all the studies cited the association was not significant.

The prevalence of HPV infection among the women who ever smoked or had a partner who ever smoked (18.18%) was higher than those who never smoked nor partner who ever smoked (15.79%). This finding was not significant even though the finding was consistent with several studies done including the one done in Kinshasa, DR Congo. In Kinshasa, DR Congo's study, those who ever smoke had prevalence of HPV infection of 16.37% and those who never smoked had prevalence of 13.15% (Sangwa-Lugoma et al., 2011).

The prevalence of HPV infection among the women who ever consumed alcohol (17.74%) was higher than those who never consumed alcohol (12.99%). Despite the fact that this finding was not significant many studies have linked alcohol to the prevalence of sexually transmittable infections.

The prevalence of HPV infection was highest among those diagnosed of hypertension (25.00%), followed by those diagnosed of sexually transmitted infection (19.05%) and then those who were not diagnosed of any of the above mentioned conditions (12.73%). The finding was not significant and since HPV infection is a sexual transmitted infection, it was expected that those diagnosed with sexually transmitted infection should have had the highest prevalence. There are associations between age and hypertension and between age and HPV infection. These linkages might have increased the prevalence among those who were diagnosed of hypertension. Thus age could have an interaction in the association between hypertension and HPV prevalence. With the HPV infection associated with the sexually transmitted infection, the finding was consistent with the studies done in Kampala, Uganda (Franceschi et at, 2008) and Kinshasa, DR Congo (Sangwa-Lugoma et al., 2011) in which those diagnosed with STDs had HPV prevalence of 3.66% as compared to those who were never diagnosed of STDs being 14.83. The decrease of HPV prevalence among those diagnosed of STDs could be due to the assertion that women diagnosed of STDs become more careful and therefore, reduce their encounter with more sexual partners. Several studies

showed that some of the antiviral therapies uses in the treatment of some STDs have effects on other viruses including HPVs. Some of the women diagnosed with STDs could have the HPV cleared as a result of treating the STDs.

The majority (72.64%) of the women in the study were aware of cervical cancer and the availability of cervical cancer screening services. This high level awareness was consistent with a study done by Amoah (2011) on the awareness of cervical cancer among University of Ghana students where about 96.3% of the participants were aware of cervical cancer. The prevalence of HPV infection among the women who were aware of cervical cancer and the availability of screening services (17.81%) was higher than those who were not aware (10.91). Less than a quarter (22.89%) of the women was aware that, only sexually active women were at risk of contracting HPV. The prevalence was highest (19.57%) among those who thought that all women, irrespective of sexual life, can be infected as compared to those who knew (15.54%) and those who did not know at all (13.33%). These findings showed that a lot of women were aware of cervical cancer but were not screening. A little more than a quarter (30.35%) of the women ever screened for HPV and cervical cancer. The prevalence among those who ever screened (16.39%) is higher than those who never screened (15.71%). A very small number of the women ever screened with the use of Papanicolaou's smear (14.93%), VIA (15.42%) and none ever used PCR (HPV-DNA). The findings were not consistent with the study done in Kinshasa, DR Congo by Sangwa-Lugoma et al. (2011). The findings of the method ever used by participants in screening even though not significant, could be interacted with where participants lived most of their lives, medical and gynaecological history. Those who live in the urban communities and have been visiting hospitals are more privileged to information on cervical cancer and the availability of screening services than those living in rural communities.

The overall prevalence of HPV 6, 11, 16, 18 and other types (detected collectively) infection was 15.92%. The prevalence of HPV infection in this study was slightly higher than the prevalence (10.7%) found in Accra by Domfeh et al.(2008) and , 12.5% in Kinshasa, DR Congo by Sangwa-Lugoma et al.(2011) . Previous cervical HPV prevalence studies in sub-Saharan Africa have generally shown relatively high prevalence with some variations, based on the sample selection and the type of HPV testing employed. Polymerase chain reaction (PCR)-based assays, similar to that employed in this study, showed HPV prevalence of 44% in Nairobi, Kenya, 40% in rural Mozambique,34% in Northwestern Tanzania, 31% in Harare, Zimbabwe, 26.3% in Ibadan, Nigeria, 18% in Dakar and Pikene, Senegal, and 15.4% in South Africa(Domfeh et al,.2008) .

In this study the highest prevalence (20.25%) was among women aged 41–60 years. the peak prevalence among women aged 41–60 years is similar to findings of a study done in Nigeria with prevalence among women aged 45-54 years being 26.0% (Thomas et al.,2004).

Of the women with HPV 6, 11, 16, 18 or other types detected, 98.51 % (29/32) had one type, 1.49 % (3/32) had two types, and none had either three or all four types. Overall prevalence of specific types was 1.00% (2/201) for HPV 6/11, 2.99% (6/201) for HPV 16, and 1.99% (4/201) for HPV 18 and 9.95% (20/201) for other HPV. This finding showed that the other genotypes detected collectively were more common in Ghana than HPV 6, 11, 16 and 18. This was not consistent with the findings that HPV 16 and 18 are the commonest types. However, among HPV 6, 11, 16 and 18, HPV 16 had the highest prevalence. This finding was consistent with a study done in United States. In an evaluation of urine specimens from women aged 18–25 years in the United States; HPV 6 and 11 were found in 2.2%, and types 16 or 18 were present in 7.8% (Dunne et al., 2013).

Other studies have found a similar prevalence of HPV 6, 11, 16, or 18 infections, and prevalence of HPV infection depends on the methods used and the population studied. A

study of sexually experienced urban females aged 13–26 years found a higher prevalence; 33.1% were positive for HPV 6, 11, 16, or 18, and 3.5% were positive for both HPV 16 and HPV 18. One prospective study found that, by 24 months, 10.4% (95% CI, 7.8–13.8) of sexually experienced young women with no baseline HPV infection had evidence of HPV type 16 infection by DNA detection. These studies, although using different methods and populations, found that few or no females had concurrent infection with all 4 types (Dunne et al., 2013).

The study found differences in trends by age group for detection of HPV 6 and 11, compared with HPV 16 and 18. Prevalence of HPV 6 and 11 infection was highest (1.56%) in the youngest age group (19-30 years), whereas HPV 16 and 18 peaked (6.33%) among women aged 40–60 years. This finding was similar to the findings in United States (Dunne et al., 2013).

The reasons for the differences in age-related trends between HPV 6 and 11 and HPV 16 and 18 are unclear but could suggest differences in type tropism or transmission dynamics.

The study found that age, education, marital status, and sexual behaviour except multiple partners were not independent predictors of HPV 6, 11, 16, or 18 infections.

Limitations of the Study

The study is subjected to some limitations. The cervical samples may not be accurately or adequately collected and this may affect the accurate assessment of the prevalence of cervical HPV 6, 11, 16, or 18 infections. The extraction of the viral DNA from the cervical specimens, if not accurately done, can affect the amplification and the subsequent detection of HPV-DNA. This is because results depend on adequate specimen collection, absence of inhibitors, and sufficient HPV-DNA to be detected.

The assessment of HPV DNA prevalence does not assess cumulative incidence. Some of the participants have screened several times before participating in this study and that can reduce the prevalence of HPV 6, 11, 16, and 18 infections, especially, among those who have been screening.

The study was also limited by sample size and time. One month was not adequate enough to collect the desired sample size for the study.

Precision of the Test

The primers used in the study were tested to ensure that there was selection of stringent reaction condition to avoid cross reactivity. The detection limit of the primers was 10 copy/reaction (10 copy/3ul DNA).

Using cytology, comparison tests were carried out on the detectable HPV. As a result, it showed over 96% matches in HSIL and 100 % matches in Squamous carcinoma (2/2).

Clinical samples were used to compare the primers with Hybrid capture II (DIGENE) and Linear assay (ROCH). As a result, they showed 95% matches for sensitivity and specificity with Hybrid capture II and 92 % matches for sensitivity / specificity with Linear assay.

CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, this study suggests a high prevalence of HPV infection among the women attending Ridge Hospital and that having multiple sexual partners or partner having multiple sexual partners is associated with human papillomavirus infection, (OR=2.68, p=0.01). This means having multiple sexual partners increases the odds prevalence of human papillomavirus infection by 2.68 times. Therefore, women who have multiple sexual partners increase their odds of contracting human paillomavirus by 2.68 times compared to women without multiple sexual partners.

The overall prevalence of HPV 6, 11, 16, 18 and other types (detected collectively) infection was 15.92%. In this study the highest prevalence (20.25%) was among women aged 41–60 years. Of the women with HPV 6, 11, 16, 18 or other types detected, 98.51 % (29/32) had one type, 1.49 % (3/32) had two types, and none had three nor all four types. Overall prevalence of specific types was 1.00% (2/201) for HPV 6/11, 2.99% (6/201) for HPV 16, and 1.99% (4/201) for HPV 18 and 9.95% (20/201) for other HPV. This finding showed that the other genotypes detected collectively were more common in Ghana than HPV 6, 11, 16 and 18 genotypes.

6.2 recommendations

Based on the findings and the conclusion of the study, the following recommendations are made:

Ghanaian women should be encouraged to have regular cervical cancer and human papillomavirus screening. Also, they should be advised against acquisition of new partners either before or after their current unions.

PCR for HPV- DNA screening should be made available and accessible to the women, since it has the best sensitivity and specificity, to detect the presence of HPV DNA earlier before the development of precancerous cells.

Vaccination against HPV should be made available and accessible to Ghanaian girls before age 18 years or before their first sex.

Vaccination against HPV should be made available and accessible to women who have tested negative to the HPV-DNA.

More studies on the prevalence of HPV specific genotypes should be encourage among students in Public Health Schools in the country. Experienced researchers should be encouraged to do more studies in this area to establish all the specific genotypes in Ghana.

Policy makers should take all the specific genotypes, especially, the predominant genotypes in the country in to consideration before the introduction of the vaccines.

REFERENCE

Abotchie, P. N., & Shokar, N. K. (2009). Cervical cancer screening among college students in Ghana: knowledge and health beliefs. *International journal of gynecological cancer: official journal of the International Gynecological Cancer Society*, *19*(3), 412.

Arbyn, M., Anttila, A., Jordan, J., Ronco, G., Schenck, U., Segnan, N., Wiener, H., Herbert, A., & Von Karsa, L. (2010). European Guidelines for Quality Assurance in Cervical Cancer Screening, Second Edition—Summary Document. *Annals of Oncology*, 21 (3), 448-458.

Arbyn, M., Castellsagué, X., De Sanjosé, S., Bruni, L., Saraiya, M., Bray, F., & Ferlay, J. (2011). Worldwide Burden of Cervical Cancer in 2008. *Annals of Oncology*, 22 (12), 2675-2686.

Banura, C. et al. (2008). Infection with Human Papillomavirus and HIV among Young Women in Kampala, Uganda.

Bernard, H., Burk, R., Chen, Z., Van Doorslaer, K., Zur Hausen, H., & De Villiers, E. (2010). Classification of Papillomaviruses (PVs) Based on 189 PV Types and Proposal of Taxonomic Amendments. *Virology*, 401 (1), 70-79.

Bhatla, N., Classen, M., Denny, L., Fagan, J., Franceschi, S., Gueye, S., Jalloh, M., Mohammed, Z., Ngoma, T., Niang, L., Prinz, C., Quek, S., Torode, J., Wiersma, S., Wittet, S., You, W., & Hausen, H. (2010). *Protection Against Cancer-Causing Infections - World Cancer Campaign 2010*. Geneva: International Union Against Cancer (UICC).

Castellsagué, X., & Muñoz N. (2003). Chapter 3: Cofactors in Human Papillomavirus Carcinogenesis—Role of Parity, Oral Contraceptives, and Tobacco Smoking. *Journal of the National Cancer Institute: Monographs*, 20 (31), 20-28.

Castellsagué, X., De Sanjosé, S., Aguado, T., Louie, K., Bruni, L., Muñoz, J., Diaz, M., Irwin, K., Gacic, M., Beauvais, O., Albero, G., Ferrer, E., Byrne, S., & Bosch, F. (2007). HPV and Cervical Cancer in the World: 2007 Report. *Vaccine*, 25 (S3), 1-241.

Clifford, G., Gallus, S., Herrero, R., Muñoz, N., Snijders, P., Vaccarella, S., Anh, P., Ferreccio, C., Hieu, N., Matos, E., Molano, M., Rajkumar, R., Ronco, G., De Sanjosé, S., Shin, H., Sukvirach, S., Thomas, J., Tunsakul, S., Meijer, C., & Franceschi, S. (2005). Worldwide Distribution of Human Papillomavirus Types in Cytologically Normal Women in the International Agency for Research on Cancer HPV Prevalence Surveys: A Pooled Analysis. *The Lancet*, 366 (9490), 991-998.

Coleman, M. A., Levison, J., & Sangi-Haghpeykar, H. (2011). HPV vaccine acceptability in Ghana, West Africa. *Vaccine*, *29*(23), 3945–3950.

Cuzick, J., Arbyn, M., Sankaranarayanan, R., Tsu, V., Ronco, G., Mayrand, M., Dillner, J., & Meijer, C. (2008). Overview of Human Papillomavirus-Based and Other Novel Options for Cervical Cancer Screening in Developed and Developing Countries. *Vaccine*, 26 (S10), K29-K41.

Denny, L., Quinn, M., & Sankaranarayanan, R. (2006). Chapter 8: Screening for Cervical Cancer in Developing Countries. *Vaccine*, 24 (S3), S71-S77.

Domfeh, A. B., Wiredu, E. K., Adjei, A. A., Ayeh-Kumi, P. F. K., Adiku, T. K., Tettey, Y., … Armah, H. B. (2009). Cervical human papillomavirus infection in Accra, Ghana. *Ghana*

medical journal, *42*(2). Retrieved from http://www.ajol.info/index.php/gmj/article/view/43596

Dunne, E., Unger, E., Sternberg, M., McQuillan, G., Swan, D., Patel, S., & Markowitz, L. (2007). Prevalence of HPV Infection among Females in the United States. *Journal of the American Medical Association*, 297 (8), 813-819.

Edwin, A. K. (2010). Is routine human papillomavirus vaccination an option for Ghana? *Ghana medical journal, 44(2).*

Franceschi, S., Herrero, R., Clifford, G., Snijders, P., Arslan, A., Anh, P., Bosch, F., Ferreccio, C., Hieu, N., Lazcano-Ponce, E., Matos, E., Molano, M., Qiao, Y., Rajkumar, R., Ronco, G., De Sanjosé, S., Shin, H., Sukvirach, S., Thomas, J., Meijer, C., & Muñoz, N. (2006). Variations in the Age-Specific Curves of Human Papillomavirus Prevalence in Women Worldwide. *International Journal of Cancer*, 119 (11), 2677-2684.

Franco, E., Mahmud, S., Tot, J., Ferenczy, A., & Coutlée, F. (2009). The Expected Impact of HPV Vaccination on the Accuracy of Cervical Cancer Screening: The Need for a Paradigm Change. *Archives of Medical Research*, 40 (6), 478-485.

Giuliano, A., Lu, B., Nielson, C., Flores, R., Papenfuss, M., Lee, J., Abrahamsen, M., & Harris, R. (2008). Age-Specific Prevalence, Incidence, and Duration of Human Papillomavirus Infections in a Cohort of 290 US Men. *Journal of Infectious Diseases*, 198 (6), 827-835.

Giuliano, A., Sedjo, R., Roe, S., Harris, R., Baldwin, S., Papenfuss, M., Abrahamsen, M., & Inserra, P. (2002). Clearance of Oncogenic Human Papillomavirus (HPV) Infection: Effect of Smoking (United States). *Cancer Causes and Control*, 13 (9), 839-846.

Goldhaber-Fiebert, J., Stout, N., Salomon, J., Kuntz, K., & Goldie, S. (2008). Cost-Effectiveness of Cervical Cancer Screening with Human Papillomavirus DNA Testing and HPV-16,18 Vaccination. *Journal of the National Cancer Institute*, 100 (5), 308-320.

Goldie, S., Gaffikin, L., Goldhaber-Fiebert, J., Gordilo-Tobar, A., Levin, C., Mahé, C., & Wright, T. (2005). Cost-Effectiveness of Cervical Cancer Screening in Five Developing Countries. *New England Journal of Medicine*, 353 (20), 2158-2168.

Hildesheim, A., & Wang, S. (2002). Host and Viral Genetics and Risk of Cervical Cancer: A Review. *Virus Research*, 89 (2), 229-240.

Keita, N. et al. (2009). HPV Infection in Women with and without Cervical Cancer in Conakry, Guinea.

Markowitz, L., Sternberg, M., Dunne, E., McQuillan, G., & Unger, E. (2009). Seroprevalence of Human Papillomavirus Types 6, 11, 16, and 18 in the United States:

Mitchell S., Ogilvie, G., Steinberg, M., Sekikubo, M., Biryabarema, C., & Money, D. (2011). Assessing Women's Willingness to Collect their Own Cervical Samples for HPV Testing as Part of the ASPIRE Cervical Cancer Screening Project in Uganda. *International Journal of Gynecology & Obstetrics* 114 (2), 111-115.

Papillomavirus Infection in Women From a Sub-Saharan African Community. National Health and Nutrition Examination Survey 2003–2004. *Journal of Infectious Diseases*, 205 (7), 1059-1067. Nigeria.

Parkin, D., & Bray, F. (2006). Chapter 2: The Burden of HPV-Related Cancers. *Vaccine*, 24 (3), S11-S25.

Sangwa-Lugoma, G. et al. (2011). Prevalence and Determinants of High-Risk Human

Schiffman, M., & Adrianza, M. (2000). Atypical Squamous Cells of Undetermined Significance (ASCUS) - Low-Grade Squamous Intraepithelial Lesion (LSIL) Triage Study: Design, Methods, and Characteristics of Trial Participants. *Acta Cytologica*, 44 (5), 726-742.

Thomas, J. et al. (2004). Prevalence of papillomavirus infection in women in Ibadan,

Tóth, A., Bánky, D., & Grolmusz, V. (2010). Mathematical Modeling and Computer Simulation of Brownian Motion and Hybridization of Nanoparticle-Bioprobe-Polymer Complexes in the Low Concentration Limit. *Nanotech*, 3, 161-164.

Trottier, H., & Burchell, A. (2009). Epidemiology of Mucosal Human Papillomavirus Infection and Associated Diseases. *Public Health Genomics*, 12 (5-6), 291-307.

Trottier, H. & Franco, E. L. (2006). Human papillomavirus and cervical cancer: Burden of illness and basis for prevention. *The American journal of managed care.*

World Health Organization (WHO), (2002a). Cervical Cancer Screening in Developing Countries. Geneva: *WHO Programme on Cancer Control, Department of Reproductive Health and Research.*

World Health Organization (WHO), (2008a). Human Papillomavirus (HPV) Vaccine Background Paper. *Canberra: Biotext Proprietary Company, Limited.*

World Health Organization (WHO), (2008b). Cervical Cancer, Human Papillomavirus (HPV), and HPV Vaccines: Key points for Policy-Makers and Health Professionals. Geneva.

World Health Organization (WHO), (2009). Human Papillomavirus Vaccines: WHO Position Paper. *Weekly Epidemiological Record*, 15 (84), 117-132.

World Health Organization (WHO), (2010). *International Agency for Research on Cancer (IARC) Handbook of Cancer Prevention, Volume 10: Cervix Cancer Screening*. Geneva: IARC.

World Health Organization (WHO). (2011). Screening is Still the "Best Buy" for Tackling Cervical Cancer. *Bulletin of the World Health Organization*, 89 (9), 621-700.

APPENDICES

Appendix I: Consent Form

Project Title: Prevalence of Human Papillomavirus among Women Attending Ridge Hospital, Accra

Principal Investigator: Alabira Iddrisu Alhassan

Qualification: Master of public health student

Tel: 0244-572440 Email: alabira.iddrisu @gmail.com

General information about the research

In Ghana, cervical cancer constitutes about 57.8% of all gynaecological cancers. It is the second most common cancer in women with an estimated incidence of 26.4 per 100,000. Human papillomaviruses (HPVs) are aetiological agents of cervical cancer. Genital HPVs are the most common sexually transmitted viruses in human. The purpose of this study is to determine the prevalence of HPV and the associated risk factors among women. The study involves answering questions from an open ended questionnaire about your demographic characteristics, medical, gynaecological and sexual history .Your response to the questions posed to you will help to achieve the purpose of this study. You will not be coerced into participating in this study, however, it will be appreciated if you could partake in it. We will be glad if you can read this consent form or have it explained to you so as to make a decision on your participation. This forms part of my assessment for the award of a master of public health degree.

Accepting to take part in this study will take about 20 minutes of your time to answer the questions attached to this form. The study will involve all women between the ages of 19 and 60 years who report to a Family Planning and HPV screening centre of Ridge Hospital for advice on family planning, counselling, breast and cervical cancer screening.

Reason for the Research

You are being asked to take part in the research to obtain data on your HPV status, sexual, medical, gynaecological history and socio demographic characteristics.

Your Part in the Research

If you agree to be part of the research, you will be interviewed and you will fill out a questionnaire that will seek for your sexual history, medical/gynaecological history socio demographic characteristics.

You are to note that some of the questions are about your private life which might be embarrassing and you may not have to answer any question if you do not want to. Cervical samples will be taken from you by a gynaecologist to be tested in the laboratory for the detection of HPV. About 286 participants will take part in this research at FPHC of Ridge Hospital.

Possible Benefits

Based on the results of the test, participants who need to go for further test or treatment would be advised accordingly.

Possible Risk/Discomfort

No additional physical, social and psychological risks are anticipated for participating in this study. What you will go through will be the same whether you got tested in this research or tested without taking part in the research as your cervical sample will be taken for Pap smear by the centre. You are allowed to withdraw from the interview anytime you wish. You are also allowed to skip answering some questions that you may find uncomfortable, even though answers to questions will be handled confidentially.

Confidentiality

We will protect information about you and your taking part in this research to the best of our ability. Your answered questionnaires and samples will be coded. These codes will only be

known by the principal investigator for the sake of finding you if need be. You will not be named in any reports. However, the staff of the FPHC of Ridge Hospital may only look at your research records for the purpose of treatment. Someone from the IRB might want to ask you questions about being in the research, but you do not have to answer them if you do not want to. A court of law could order medical records shown to other people, but that is unlikely.

All the samples and questionnaires will be locked in a safe place by the principal investigator and he shall be the only person who will have access to the safe.

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

Compensation

Eligible persons who consent to participate in the study will not be given any monetary or non monetary compensation.

Participant's Consent

I have read or someone has explained to me all the necessary details I need to know concerning this study. I have therefore decided without any coercion to participate in this study. However by signing or thumb printing this consent form, I am not waiving off any of my personal rights.

Signature .....

Right/left thumb print

Contact Information about This Study

In case you need further clarification regarding this study, kindly contact any of the following;

Miss Nana Abena Kwaa Addai-Donkor (0244712919), Administrator, GHS ethical review committee.

Alabira Iddrisu Alhassan. Tel: 0244-572440Email: alabira.iddrisu @gmail.com

Appendix II: Questionnaire

Project Title: Prevalence of Human Papillomavirus among Women Attending Ridge Hospital, Accra

Questionnaire on sociodemographic characteristics, medical, gynaecological and sexual history of women aged 19 to 60 years attending FPHC of Ridge Hospital

This questionnaire will not be administered to males and any female below the age of 19 years.

Date of interview .....

Time of interview.....

ID of respondent……

Tel no. …….....….. .....

Interviewed by ……

Please tick

Sociodemographic Characteristics

1. Age ……..

2. Date of birth ……..

3. Educational Background

No formal education [ ] Primary School [ ]

JSS/Middle School [ ] Secondary School [ ]

Tertiary Education [ ]

4. Occupation: Housewife [ ] Self Employed [ ]

Civil Servant [ ] Manual/Menial [ ] Unemployed [ ]

5. Income per Month: < GH¢ 100 [ ] 100-500 [ ]

600-1000 [ ] More Than 1000 [ ]

6. Where have you lived most of your life? Village [ ] Town [ ] City [ ]

7. What type of house do you stay in? No Permanent Residence [ ]

Self contained house [ ]

Compound House [ ]

Others [ ]

8. Religion: Christian [ ] Muslim [ ] African Tradition [ ] Others [ ]

9. Ethnicity: Akan [ ] Dagbani [ ] Ewe [ ] Ga [ ] Hausa [ ] Others [ ]

Sexual History

10. At what age did you have your first sexual contact? [ ]

11. How many sexual partners do you have? [ ]

12. Did you have a partner before meeting the current partner? Yes [ ] No [ ]

13. What is you marital Status? Single [ ] Married/Cohabited [ ] Divorced/Widowed/Separated [ ]

14. Have you ever used modern contraception? Yes [ ] No [ ]

15. Does your partner have other partners? Yes [ ] No [ ]

16. Did your partner have a partner before meeting you? Yes [ ] No [ ]

17. Did your partner have a partner after meeting you? Yes [ ] No [ ]

18. Does Your Partner Use a Condom? Yes [ ] No [ ]

Gynaecological History

19. How many pregnancies have you had?

20. How many children do you have?

Medical History

21. Do you smoke or have you ever smoked? Yes [ ] No [ ]

22. Does your partner smoke or has your partner ever smoked? Yes [ ] No [ ]

23. Do you drink alcohol? Yes [ ] No [ ]

24. Have you ever taken alcohol? Yes [ ] No [ ]

25. Have you ever been diagnosed of the following?

(1) Hypertension [ ] (2) Cancer [ ] (3) Tuberculosis [ ] (4) Genital Warts [ ]

(5) HIV [ ] (6) Diabetes [ ] (7) Sexual transmitted infections [ ] (8) Non of the above [ ]

Knowledge of the Causes of Cervical Cancer

26. Have you ever heard of cervical cancer? Yes [ ] No [ ]

27. Who do you think are at risk of suffering from cervical cancer? All women [ ]

Women Who Have Had Sex [ ]

. Do not know [ ]

28. Have you heard of screening for cervical cancer? Yes [ ] No [ ]

29. Have you ever screened for cervical cancer? Yes [ ] No [ ]

30. Which method was used: Pap smear [ ] VIA [ ]

PCR [ ] Do not know [ ]