PREVALENCE OF HUMAN PAPILLOMAVIRUS IN ASSOCIATION WITH CD4+ COUNT AMONG HIV POSITIVE WOMEN ATTENDING BARAU DIKKO TEACHING HOSPITAL IN KADUNA, NIGERIA

BY

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DECLARATION

I hereby wish to declare that the work in this Dissertation titled "Prevalence of Human Papillomavirus in Association with CD4+ Count among HIV Positive Women Attending Barau Dikko Teaching Hospital in Kaduna, Nigeria" was performed by me in the Department of Microbiology, under the supervision of Prof. C.M.Z. Whong and Dr. E.E. Ella. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

_____ _____

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CERTIFICATION

This Dissertation entitled "Prevalence of Human Papillomavirus in Association with CD4+ Count among HIV Positive Women Attending Barau Dikko Teaching Hospital in Kaduna, Nigeria" meets the regulation governing the award of the degree of Masters of Science of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This Dissertation is dedicated to Almighty Allah for his guidance and protection throughout this program.

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ABSTRACT

Human papillomavirus (HPV) is the cause of 90-95% of squamous cell cancers. Persistent infection with high risk HPV can lead to development of cervical cancer. This study was conducted to determine the prevalence of HPV immunoglobulin G (IgG) antibodies and its association with CD4+ count among HIV positive women attending Barau Dikko Teaching Hospital in Kaduna, Nigeria. The study was descriptive, cross-sectional and experimental combining the use of structured questionnaire and as well as analysis of whole blood for CD4+ count and serum for IgG antibodies to HPV using Beckton Dickinson FACS Count and ELISA accordingly.The prevalence of IgG antibody to HPV was found to be 21.9% (59/270) in 270 HIV positive women who came for their CD4+ count in Barau Dikko Teaching Hospital in Kaduna Metropolis, Kaduna State, Nigeria. Women in the age group 20-29 had the highest prevalence of 38.9% (21/54). In this study, IgG antibodies to HPV was predominant in the younger than the older women who had their first sexual intercourse in the age bracket 10-19 years and had the highest prevalence 23.0% (31/135) of HPV. The result varied with the socio-demographic factors but was only significantly associated with educational status, age, religion and women having children ($P< 0.05$). The results also insignificantly varied with the reproductive and sexual behavior of the women. The prevalence of HPV among the HIV positive women with CD4+ count greater than 200cells/mm^3 was higher 22.0% (53/241) compared to 21.4% (6/28) obtained among those with CD4+ count less than 200cells/mm^3.Further studies on the prevalence of high risk serotypes of HPV among HIV positive and negative women as well as HPV related malignancies among women is recommended.

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CHAPTER ONE

1.0 INTRODUCTION

Human papillomavirus (HPV) is a non-enveloped DNA virus, belonging to Papillomaviridae family that is capable of infecting humans (Cutt *et al.,* 2007). Approximately hundred types of HPV have been identified with at least fourty of which infect the genital area (CDC, 2015). Oncogenic, high-risk HPV infection (HPV typesb16 and 18) cause most cervical, penile, vulvar, vaginal, anal and oropharyngeal cancers and precancers (CDC, 2015), whereas nononcogenic, low-risk HPV infection (HPV types 6 and 11) causes genital warts and recurrent respiratory papillomatosis (CDC, 2015). More than 30-40 types of human papillomavirus are typically transmitted through sexual contact and infect the anogenital region leading to increased risk of development of cancer (Smits *et al.,* 2005). HPV-16 is the most common HPV infection in cervical cancer (Clifford *et al.,* 2003).

Risk factors associated with the infection of HPV include; heterosexuality, promiscuity, smoking, high parity, sexual indulgence, infection with other sexually transmitted diseases, prolonged use of contraceptives, genetic disorders and dietary factors (Thomas *et al*., 2004). Cervical cancer is a malignant neoplasm arising from cells originating in the cervix uteri. One of the most common symptoms of cervical cancer is abnormal vaginal bleeding, but in some cases there may be no obvious symptoms until the cancer has progressed to an advanced stage (Kumar *et al*., 2007). Human papillomavirus infection appears to be a necessary factor in the development of almost all cases (90 - 95%) of cervical cancer (Canava and Doshi, 2000; Kumar *et al*., 2007). Early HPV infections may be accomplished by mild changes in the epithelium and most HPV infections of the cervix are asymptomatic (Moscicki *et al*., 2006). Most HPV

infections in young females are temporary and have little long-term significance. Seventy percent (70%) of infection may disappear in one year and 90% in 2 years (Goldstein *et al.,* 2009). However, in about 5-10% of infected women, there is high risk of developing precancerous lesions of the cervix due to persistent infection with the virus, which can progress to invasive cervical cancer and this process usually takes 15-20 years (Kahn, 2009). Persistent infection following acquisition of high-risk HPV (hr HPV) is an important early precursor event in the carcinogenic progression to cervical cancer (Bosch *et al.,* 2002). It is generally defined by continued detection of DNA of the same HPV type in cervical scrapings (Ho *et al*., 1995).

Human Papillomavirus is known to increase the incidence of cervical cancer in HIV/AIDS patients (Ferlay *et al.,* 2007). The impaired cell mediated immunity due to HIV infection results in the body's inability to clear high risk human papillomavirus (hr HPV) leading to the persistence of the virus in the cervix and eventual transformation of infected cervical cells to precancerous and cancerous lesions (Ngandwe *et al.,* 2007). With the diverse HIV epidemic in sub-Saharan Africa, it is essential to consider how it may affect HPV related cervical diseases including cervical cancer (Louie *et al.,* 2009). Studies from Southern and Eastern Africa have noted a strong association between HIV, HPV co-infection and the development of genital cancers (Clifford *et al.,* 2003; Clifford *et al.*, 2006; Blossom *et al.,* 2007; Anorlu, 2008).

CD4+ cells or T-cells are a collective immune system that play major role in protecting the body from infection. They send signals to activate the body's immune response when they detect "intruders," like viruses or bacteria (AIDS.gov, 2014). A CD4+ count is a measure of the number of CD4+ cells in a sample of the blood. It is an important indicator of how well the immune system is working (AIDS.gov, 2014). The CD4+ count of a healthy adult/adolescent ranges from 500-1,200 cells/mm^3. A very low CD4+ count (less than 200 cells/mm^3) is one of the ways to

determine whether a person living with HIV has progressed to stage 3 infection (Acquired Immunodeficiency Syndrome) (AIDS, 2014).

1.1 Statement of the Research Problem

Worldwide, cervical cancer is the fourth most common cancer in women, with an estimated 266,000 deaths and 528,000 new cases in 2012 (WHO, 2015). National Cervical Cancer Coalition (NCCC) in 2016 reported that, it was estimated by American Cancer Society in 2015 that worldwide, there were 12900 cases of cervical cancer, and 4100 deaths per year. Approximately Eighty percent (80%) of these new cases come from developing nations and as such it is the leading cause of cancer associated death in women in Nigeria and developing countries (Kent, 2010; Okolo, *et al.,* 2010). In developing countries, complications in immunocompromised HIV patients due to HPV and other sexually transmitted diseases (STIs) are a major cause of mother and child mortality and morbidity during pregnancy (Mullick *et al.,* 2005). In sub-Saharan Africa, the cervical cancer rates are on the rise, paralleling the HIV epidemic (Blossom *et al.,* 2007; Anorlu, 2008).

Women infected with HIV have consistently been shown to have higher prevalence of HPV infection, persistent infection with HPV, infection with multiple types of HPV, and cervical cancer precursors than HIV-uninfected women (Harris *et al.,* 2005; Denny *et al.,* 2008; Singh *et al.,* 2009). The HPV type distribution varies among geographical regions but worldwide, HPV 16 and 18 are the most common HPV types in most cervical cancer (Center for Disease Control (CDC), 2015).

1.2 Justification

Virtually all cervical cancer cases (99%) are linked to genital infection with HPV and it is the most common viral infection of the reproductive tract (WHO, 2015). Human papillomavirus infection appears to be a necessary factor in the development of almost all cases (90%) of cervical cancer (Canava and Doshi, 2000; Kumar *et al*., 2007). High-risk types of HPV are identified in nearly all carcinomas of the cervix and the relative risk of cervical cancer associated with infection with high-risk types of HPV is higher than the risk of lung cancer associated with smoking (Walboomers *et al.*, 1999).

The observed rise in cervical cancer rate in the sub-Sahara Africa has been linked to the chronic deviations in the immune systems due to HIV infection (Strickler, 2005; Jong *et al* ., 2009; Luquea *et al.,* 2010). The increased incidence, prevalence and persistence of HPV infection in HIV-positive women is attributed to increased susceptibility, decreased ability to clear infection due to impaired cell-mediated immunity, and reactivation of latent HPV infection associated with immunosuppression (Palefsky, 2009).

Infection with high-risk types of HPV (hrHPV) is etiologically associated with the development of cervical cancer, and is associated with other anogenital tract cancers (anus, vulva, vagina, penis) and a proportion of oropharyngeal cancers (Walboomers *et al.*, 1999; Parkin *et al.*, 2008). The expected increase in anogenital cancers in HIV-positive individuals had recently become apparent, with increases in cervical cancer and anal cancer being well documented (Chaturvedi *et al.*, 2009; Silverberg *et al.*, 2011; Lacey, 2016).

1.3 Aim of Study

The aim of this research was to determine the prevalence of Human papillomavirus infection in HIV positive women attending Barau Dikko Teaching Hospital in Kaduna metropolis, Nigeria.

1.4 Specific Objectives

The specific objectives of this research were to:

1. investigate the risk factors associated with HPV in HIV positive women attending Barau Dikko Teaching Hospital in Kaduna using structured questionnaire.

2. determine the CD4+ count of the HIV positive women studied above and its correlation with their HPV status.

3. determine the prevalence of HPV in HIV positive women attending Barau Dikko Teaching Hospital in Kaduna using ELISA.

1.5 Research Hypothesis

Ho: There is no HPV infection among HIV positive women attending Barau Dikko Teaching Hospital in Kaduna metropolis, Nigeria.

Ha: There is HPV infection among HIV positive women attending Barau Dikko Teaching Hospital in Kaduna, Nigeria.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical Perspective of Human papillomavirus and Cervical Cancer

In 1976 Harald zur Hausen published the hypothesis that human papilloma virus plays an important role in the cause of cervical cancer. In 1983 and 1984 zur Hausen and his collaborators identified HPV16 and HPV18 in cervical cancer. In 1972, the association of the human papilloma viruses with skin cancer in epidermodysplasia verruciformis was proposed by Stefania Jabłońska in Poland. In 1978, Jabłońska and Gerard Orth at the Pasteur Institute discovered HPV-5 in skin cancer (WHO, 2007). The link between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold zur Hausen, a German virologist. Since then, the link between high-risk HPV types and cervical squamous cell carcinoma has become well known. In 1996, the World Health Association recognized HPV as an important cause of cervical cancer. HPV has been implicated in 99.7% of cervical squamous cell carcinoma cases worldwide (Walboomers *et al.,* 1999).

2.2 Virology and Properties of Human papillomavirus.

2.2.1 The Human papillomavirus

Human papillomavirus as shown in Figure 2.1 is a member of the *Papovaviridae* family consisting of a small, nonenveloped virus, with a diameter of 55nm. It has an icosahedral capsid composed of 72 capsomers, which contain at least two capsid proteins, L1 (major) and L2 (minor) (Favre, 1975). Each capsomer is a pentamer of the major capsid protein. Each virion capsid contains several copies (about 12 per virion) of the minor capsid protein (Favre, 1975).

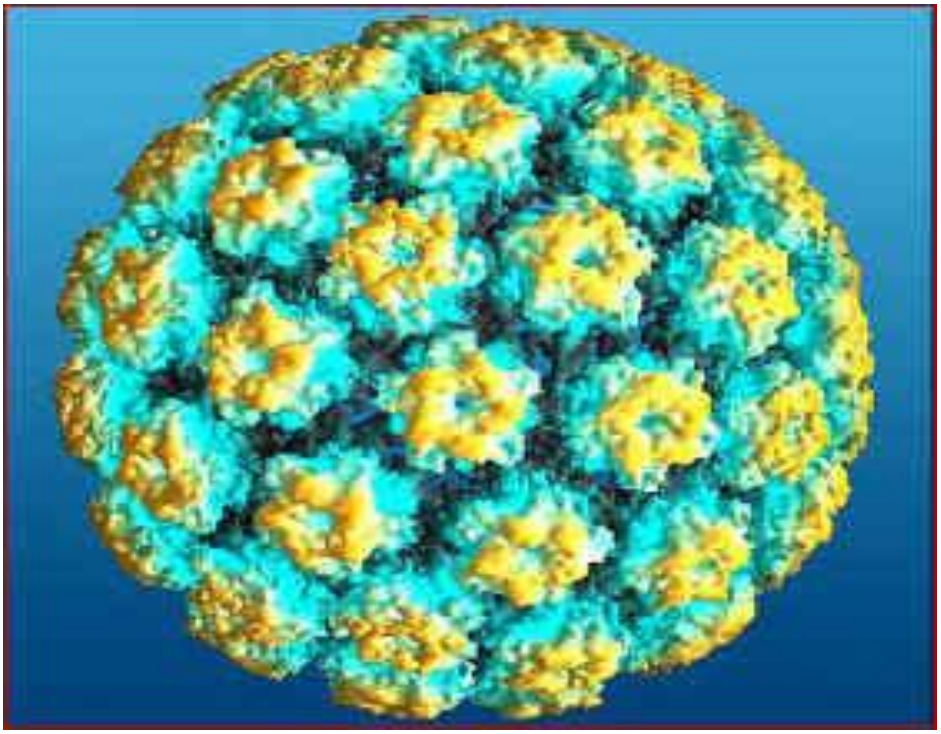


Figure 2.1: The figure above shows the structure of Human papillomavirus capsid

Adapted from;

Zheng *et al.*, (2006).

The HPV genome consists of a single molecule of double-stranded, circular DNA containing approximately 80000 base pairs associated with histones (Munoz *et al.,* 2006). The genome is functionally divided into three regions. The first is a noncoding upstream regulatory region (URR). This region contains the p97 core promoter along with enhancer and silencer sequences that regulate DNA replication by controlling the transcription of the "early" and "late" regions (Munoz *et al.,* 2006). The URR region also contains the highest degree of variation in the viral genome. The second is the "early" region,which include the genes E1, E2, E3, E4, E5, E6, E7 and E8. This region is involved in viral replication and oncogenesis (Favre, 1975). Expression of the early gene products determines whether an HPV infection is active or latent, or leads to malignant transformation. The third is the "late" region, which encodes the L1 and L2 structural proteins for the viral capsid (Favre, 1975).

2.2.2 Viral Proteins (genes) of HPV and their Functions

Viral proteins also known as genes are categorized into eight early genes comprising of E1, E2, E3, E4, E5, E6, E7, E8 and two late genes known as L1 and L2 as shown in Figure 2.2 below. The E1 genes is functions in viral replication, E2 is for modulation of transcription and replication while the function of E3 is unknown. The E4 gene functions in productive viral infections, E5 possess transformation properties, E6 servers as oncoprotein which interacts with p53 protein. The E7 is also an oncoprotein which interacts with pRb protein while E8 gene function is unknown. The L1 gene functions as the major capsid protein while the L2 gene functions as the minor capsid protein (Zheng *et al.,* 2006).

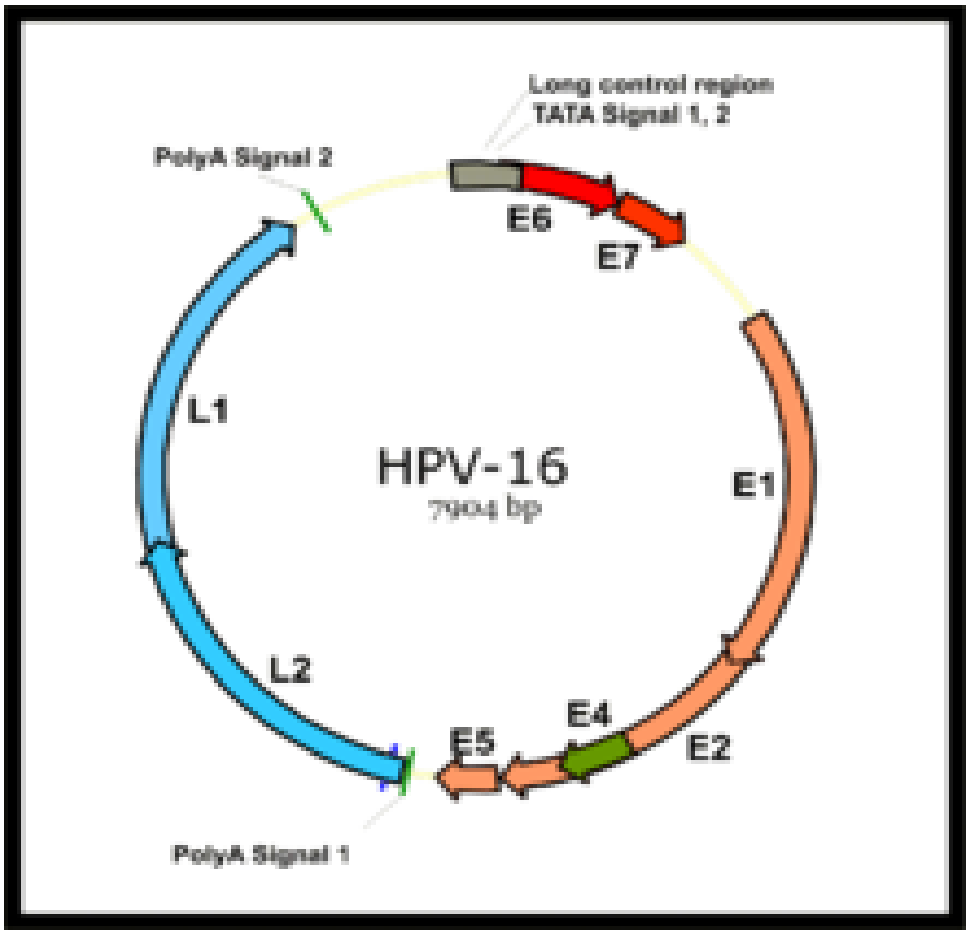


Figure 2.2: The figure above shows Genome organization of Human papillomavirus type 16, one of the subtypes known to cause cervical cancer. (E1-E7 early genes, L1-L2 late genes: capsids)

Adapted from: Zheng *et al* (2006).

2.3 Epidemiology of Human Papillomavirus

Cancer of the cervix is the commonest cancer found among women in the developing world, where more than 80% of all cases are diagnosed. In 2012, 528,000 new cases of cervical cancer and 266,000 deaths have been reported (WHO, 2015).

Munoz *et al.* (2003), pooled data from 11 case-control studies involving 1918 women with histologically confirmed squamous cell carcinoma of the cervix and 1928 control women. The pooled odds ratio for cervical cancer associated with the presence of any HPV was 158.2 (95% CI: 113.4- 220.6). On the basis of the pooled data as shown in Table 2.1, fifteen HPV types were classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82) and are considered carcinogenic; 3 were classified as probably high-risk types (26,53 and 66); and 12 were classified as low-risk types (6,11,40,42,43,44,54,61,70,72,81 and CP6108) (Munoz *et al.*, 2003).

In a meta-analysis of HPV types found in invasive cervical cancers worldwide, data on a total of 14,595 cases of invasive cancer (which included squamous cell carcinomas, adenocarcinomas and adeno-squamous carcinomas) confirmed the high prevalence of HPV in cervical cancers in different regions of the world, with HPV 16 (52 - 58%) and 18 (13 - 22%) being the commonest (Smith *et al.*, 2007). However, more than 16 other types of HPV were also associated with cervical cancer, of which types 31, 33, 35, 45, 52 and 58 were the most prevalent. Further, HPV type 16 was more prevalent in squamous carcinomas and HPV type 18 more prevalent in adenocarcinomas of the cervix (Smith *et al.*, 2007). Overall, HPV prevalence differed little between geographical regions (86-94%) but was low compared to the almost 100% prevalence in studies that have used the most sensitive methods of detection for HPV (Smith *et al.*, 2007).

Overall HPV prevalence in High Squamous Intraepithelial Lesion (HSIL) (7,094 cases of HSIL underwent HPV DNA typing) was 85%, ranging from 78% in Asia, to 88% in Europe.

Combined HPV 16/18 prevalence in all HSIL cases was 52%, with HPV 16 being the predominant type of HPV from all continents studied, varying from 34% in Asia to 52% in Europe. There is good evidence that HPV infection precedes the development of cervical cancer by a number of decades and that persistent infection with HPV is necessary for the development of and progression of pre-cancerous lesions of the cervix, either to higher grades of pre-cancerous disease or to cancer (Liaw *et al,* 1999; Nobbenhuis *et al.,* 1999; Yitalo *et al.,* 2000). Other factors aside from HPV have been investigated and found to be associated with cervical cancer and its precursors and this includes long-term use of oral contraceptives, tobacco smoking, co-infection with other sexually transmitted agents such as herpes simplex 2, Chlamydia trachomatis and HIV (Monera *et al.,* 1995 and Ho *et al.,* 1999).

The Collaborative Group on Epidemiological Studies of Cervical Cancer (ICESC) in 2007, reanalysed data from 24 epidemiological studies worldwide to investigate the association between cervical cancer and contraceptive use. Individual data for 16,573 women with cervical cancer and 35,509 women without cervical cancer were reanalysed. They found that among current users of oral contraceptives, the risk of invasive cancer increased with duration of use (relative risk for 5 or more years use *vs* never use was 1.90 (95% CI: 1.69-2.13). The risk declined after use ceased and by 10 or more years had returned to that of never users (ICESC, 2007). The effects of smoking have been studied in a number of case-control studies, and they

show a moderate and statistically significant association with cervical cancer (Monera *et al.,* 1995; Ho *et al.,* 1999).

2.3.1 Prevalence of Human Papillomavirus in HIV positive women

A recent analysis by Ezechi *et al.* (2014) found that, the prevalence of hr HPV and multiple hr HPV infection were found to be significantly higher in HIV positive women (24.6% and 8.2%) than in HIV negative women (15.9% and 3.9%) respectively (OR: 1.7; 95% CI: 1.1-3.1, OR: 2.6; 1.3-5.3). However statistically significant association with HIV infection was noted only for hr genotypes HPV 35 and 16 after controlling for confounders. Women infected with HIV were 3.6 and 3.1 times likely to be infected with HPV 35 (OR: 3.6; 95% CI: 1.4-6.9) and 16 (OR: 3.1; 95% CI: 1.3-6.3) respectively than HIV negative women (Ezechi *et al.,* 2014). The reported higher hr HPV prevalence in HIV infected women noted in this study were similar to previous observations from other regions of Africa, south America and Europe connoting that HIV infected women are at increased risk of hr HPV infection (Blossom *et al.,* 2007; Gray *et al.,* 2010; Ferrand *et al.,* 2010). The observed increase in prevalence has been attributed to HIV related immunosuppression (Ngandwe *et al.,* 2007; Piras *et al.,* 2011).

Strickler and colleagues (2005) confirmed in two separate studies an increased prevalence of HPV, persistence and decreased resolution of HPV infection in HIV positive women compared to HIV negative women (Stricker *et al.,* 2005; De Vuyst *et al.,* 2008). Clifford and colleagues (2003) showed in their metaanalysis involving 20 publications that apart from viral types, immune-suppression of HIV infection significantly increased the risk for HPV infection and invasive cancer (Palefsky *et al.,* 1999). In addition, the reported more frequent multiple HPV

types in HIV-positive women was consistent with previous reports (Clifford *et al.,* 2003; Clifford *et al.,* 2006; Louie *et al.,* 2009), and may be attributable to the common mode of transmission of HPV and HIV; persistence of HPV as a result of the inability to clear HPV infections; as well as reactivation of latent HPV infections (Palefsky *et al.,* 1999).

The higher prevalence of hr HPV infection in HIV positive women with CD4+ count <200 cells/mm3 and significantly lower prevalence in those with CD4+ count >500 cells found in their study further confirms the results of several studies that have consistently shown a higher prevalence (Ezechi *et al.,* 2014). Another meta-analysis by Li *et al.* (2011) found that HPV16 and 18 (the hrHPV types targeted by the current prophylactic HPV vaccines) are found in 57% and 16% of cervical carcinomas, respectively, in the general female population worldwide. In a recent cross-sectional study of women at high risk for STIs from Rwanda, prevalence of any HPV was 47% in HIV-negative women (mean age 25 years) compared to 72% in HIV-positive women (median age 27 years) (Veldh``uizen *et al.,* 2011). The increased incidence, prevalence and persistence of HPV infection in HIV-positive women is attributed to increased susceptibility, decreased ability to clear infection due to impaired cell-mediated immunity, and reactivation of latent HPV infection associated with immunosuppression (Palefsky, 2009).

Genital HPV infection is the most common sexually transmitted infection in the world although detection of HPV types by polymerase chain reaction (PCR) assays varies greatly with age and by geography (Clifford *et al.,* 2005), randomly selected women from 11 countries for HPV DNA testing using GP5+/6+ PCR analysis. Women aged 15-74 years who did not have cytological abnormalities were included (n = 15 613). They found that age standardized prevalence varied

nearly 20 times between different populations: from 1.4% in Spain, to 25.6% in Nigeria. Overall, age standardized HPV prevalence was five times higher in sub-Saharan Africa than in Europe with intermediate prevalence in South America and Asia. In terms of types, HPV type 16 was the commonest type in all regions except in sub-Saharan Africa, where HPV type 35 was equally common. Sanjose *et al.* (2007) performed a meta-analysis of 157879 women with normal cytology (from 78 studies) who underwent HPV DNA testing. The prevalence of HPV was estimated to be 10.4% (95% CI: 10.2-10.7) globally and the prevalence was higher in less developed countries overall (15.5%) compared to more developed countries (10.6%). Corresponding prevalence estimates for Africa were 22.1%, Central America and Mexico 20.4%, Northern America 11.3%, Europe 8.1% and Asia 8.0%. Age specific prevalence was possible to estimate from 44 studies, and HPV prevalence was shown to be highest in women younger than 34 years, with a decrease in the 35-44 year group. There was as second peak from age 45-54 years in all regions except Asia (Clifford *et al.,* 2005).

In this same analysis, 48 studies provided HPV-type specific prevalence data in women with normal cytology (Sanjose *et al.,* 2007). The five most common types of HPV were HPV 16 (2.5%), HPV 18 (0.9%), HPV 31 (0.7%), HPV 58 (0.6%) and HPV 52 (0.6%). The authors estimated that the overall burden of HPV infection from the age-specific adjusted prevalence in cytologically normal women to be 291 million women infected with HPV of whom 23.3% were estimated to be infected with HPV 16 and 8.5% with HPV 18. HPV infection is also very common in men although it has not been studied as extensively in men as in women (Partridge *et al.,* 2007).

2.3.2 Risk Factors

Risk factors for HPV infection are related to sexual behaviour, including the number of sex partners, lifetime history of sex partners, and the partners' sexual history. Most studies suggest that young age (less than 25 years) is a risk factor for infection. Results of epidemiologic studies are less consistent for other risk factors, including young age at sexual initiation, inconsistent condom use, number of pregnancies, genetic factors, smoking, lack of circumcision of male partner, and oral contraceptive use (Winer *et al.,* 2003).

2.3.3 Transmission

Human papillomavirus infections are transmitted through sexual contact (WHO, 2015). Genital HPV infection is the most common sexually transmitted infection in the world (Clifford *et al.,* 2005). HPV is transmitted by direct contact, usually sexual, with an infected person. Transmission occurs most frequently with sexual intercourse but can occur following non-penetrative sexual activity (Koutsky and Kiviat, 1999).

Studies of newly acquired HPV infection demonstrate that infection occurs soon after onset of sexual activity. In a prospective study of college women, the cumulative incidence of infection was 40% by 24 months after first sexual intercourse. HPV 16 accounted for 10.4% of infections (Winer *et al.,* 2006). Genital HPV infection also may be transmitted by nonsexual routes, but this appears to be uncommon. Nonsexual routes of genital HPV transmission include transmission from a woman to a newborn infant at the time of birth (Winer *et al.,* 2006).

2.4 Pathogenesis of Human Papillomavirus

Human papillomaviruses (HPVs) are small, non-enveloped, double-stranded DNA (dsDNA) viruses (Stanley, 2016) that infect squamous epithelial cells (Del Vecchio *et al.,* 1992) and are

responsible for benign warts of the hands, genitals and the larynx (Hughes and Romanos, 1993). To date, as many as 120 different types of HPVs have been identified, two-thirds of which infect cutaneous membranes and one-third of which infects mucosal membranes. Based on their ability to cause malignant carcinomas, the HPVs that target the mucosa can be categorized into the: high-risk type (HPV type 16, 18, 31) and the low-risk (HPV type 6, 11) type (Kajitani, 2012). The high-risk HPVs cause intraepithelial neoplastic lesions which can progress to cervical cancer, whereas the low-risk HPVs are responsible for genital warts (Condyloma acuminata) and express a rarity for progression to cancer (Del Vecchio *et al.*, 1992).

2.4.1 Natural History of Cervical Cancer

The pathogenesis of cervical cancer is initiated by HPV infection of the cervical epithelium during sexual intercourse. Even though a high percentage of sexually active young women are exposed to HPV infections, only a very small percentage go on to develop cervical cancer (Elfgren *et al.,* 2000). Several studies have suggested that most women successfully clear the HPV infection, presumably through the action of a competent immune system. Approximately, 90% of lesions regress spontaneously within 12 to 36 months (Chua and Hjerpe, 1996).

Other factors such as genetic predisposition, frequency of reinfection, intratypic genetic variation within HPV type, coinfection with more than one HPV type and hormone levels may also influence the ability to clear an HPV infection. The evidence for the importance of the host immune system in preventing the development of cervical disease comes from the analysis of HPV infections in human immunodeficiency virus (HIV)-positive women. HPV infections with high-risk viral types, persistence of HPV infection and the presence of squamous intraepithelial

lesions are more common within this immunocompromised group than in immunocompetent women (Cubie *et al.,* 2002). The host cellular immune response is mediated by T cells and requires the interaction of viral epitopes with histocompatibility class I molecules (Ostor, 1993). A humoral immune response also develops, but local levels of HPV-specific immunoglobulin G (IgG) and IgA in tissue do not correlate with clearance of virus (Ostor, 1993). However, systemic levels of HPV-specific IgA have been correlated with virus clearance. In contrast, systemic levels of HPV-specific IgG have been detected more frequently in patients with persistent HPV infection (Ostor, 1993).

The natural history of cervical cancer is a continuous disease process that progress gradually from mild cervical intraepithelial neoplasia (CIN) to more severe degrees of neoplasia (CIN 2 or CIN 3) and finally to invasive cancer (Holowaty *et al.,* 1999). It is plausible that high-risk HPV infection occurs early in life, may persist, and in association with other factors promoting cell transformation, may lead to a gradual progression to more severe disease (Ho *et al.,* 1995). Mild and moderate dysplasias are associated with continued viral replication and virus shedding, and most of these lesions spontaneously regress.

Progression to high-grade lesions (CIN 2/3) and ultimately invasive cancer is usually associated with conversion of the viral genome from an episomal form to an integrated form, along with inactivation or deletion of the E2 region and expression of the E6/E7 product genes. Some investigators have correlated HPV type with different degrees of CIN and have suggested that CIN 1 and CIN 2/3 are distinct processes, with CIN 1 indicating a self-limited sexually transmitted HPV infection and CIN 2 or CIN 3 being the only true cervical cancer precursor

(Holowaty *et al.,* 1999). Progression to cancer generally takes place over a period of 10 to 20 years. Some lesions become cancerous more rapidly, sometimes within two years (Holowaty *et al.,* 1999).

2.4.2 Viral Replication

The HPV replication begins with infection of stem cells in the basal layer of the epithelium. After the entry in the cells, the virus requires the expression of E1 and E2 genes to maintain a low number of copies of genome. These proteins bind to the viral origin of replication and recruit cellular DNA polymerases and other proteins necessary for DNA replication (Hamid *et al*., 2009). In the suprabasal layer, the expression of genes E1, E2, E5, E6 and E7 contributes to the maintenance of the viral genome and induces cell proliferation , increasing the number of HPV infected cells in the epithelium, resulting in a higher number of cells that will eventually produce infectious virions (Hamid and Gston, 2009; Lazarczyk *et al*., 2009).

In the more differentiated cells of this same layer of the epithelium occurs the activation of differentiation-dependent promoter and maintenance of gene expression E1, E2, E6 and E7. Furthermore, there will be activation of the expression of E4 gene, whose product will induce amplification of the viral genome replication, greatly increasing the number of virus copies per cell, at the same time that occurs the expression of genes L1 and L2 (Nakahara *et al*., 2005; Lazarczyk *et al*., 2009). In the granular layer, the products of late genes, the major and minor proteins of the viral capsid, L1 and L2 respectively, gather to assembly of the viral capsids and formations of virions, which reach cornified layer of the epithelium and are released (Lazarczyk *et al*., 2009). For a better understanding, the life cycle of HPV was divided into two parts: a maintenance phase and differentiation-dependent phase (Bodily and Laimins, 2011).

2.4.2.1 Maintenance phase

HPV virions infect cells in the basal epithelial layer that become exposed through microlesions. The viral capsid binds initially to the basal cell layer and infection occurs when activated keratinocytes move into the wound, to the upper layers of the epithelium (Kines *et al*., 2009). HPV genomes replicate in the nucleus of the basal cell layer, where the viral replication is considered nonproductive and the virus establishes itself as a low-copynumber episome by using the host DNA replication machinery (Moody & Laimins, 2010). In this way, viral proteins are expressed at very low levels in undifferentiated cells, and this contributes to immune avasion and persistence (Bodily and Laimins, 2011).

The maintenance of the viral episome in basal cells is the basic function of the early or maintenance phase of the viral cycle. The expression of E6, E7, E1, and E2 are necessary for continued episomal maintenance. E1 and E2 cooperate to initiate viral DNA replication, whereas E6 and E7 modulate cell-cycle regulators to maintain long-term replication competence (Conger *et al*., 1999). The E2 protein is probably a major regulator of this process because it is able to make both positive and negative control of the early viral promoter that regulates expression of E6, E7, and E1 as well as E2 itself (Steger *et al*., 1997).

Following this establishment phase, viral DNA is replicated coordinately with host cell chromosomes, and virus genomes are distributed to the daughter cells. However, in the differentiated keratinocytes of the suprabasal layers of the epithelium, the virus switches to a rolling-circle mode of DNA replication, amplifying its DNA to a high copy number, synthesizing capsid proteins, and assembling the viral particle (Flores *et al*., 1999).

Human papillomavirus replication begins when the host cell factors interact with the LCR region of the HPV genome and begin the transcription of the early viral genes, highlighting the E6 and E7. The viral E6 and E7 gene products deregulate the cell cycle, subverting the cell growth regulatory pathways and modifying the cellular environment in order to facilitate viral replication in a cell that is terminally differentiated and has exited the cell cycle (Syrjânen and Syrjânen, 1999)

2.4.2.2 Differentiation-dependent phase

During the maintenance phase in undifferentiated cells, viral proteins are expressed in extremely low levels. However, when HPV-infected cells leave the basal layer, they undergo differentiation and high levels of viral proteins synthesis are induced. This restriction of viral protein synthesis to highly differentiated cells delays the expression of viral antigens to locations less susceptible to the host immune response (Frazer, 2009).

This compartimentalization of gene expression by HPVs constitutes an important strategy to sustain long-term infection, but it creates some problems for the virus. To solve this, the virus forces the cell to remain active in the cell cycle, enabling productive replication in differentiating cells. The viral protein E7 is responsible for maintaining the replication competence in differentiated cells and this is accomplished in part by inactivation of pRB family members (Münger *et al*., 2004). The activation of the late viral promoter in response to host-cell differentiation occurs in the vicinity of the spinous epithelial layer and is responsible for high levels of viral protein expression. As a result, the virus copy-number amplifies from 50-200

copies to several thousands of copies per cell. The viral proteins E1, E4, and E5 contribute to the activation of late viral functions upon differentiation (Wilson *et al*., 2005; Fehrmann *et al*., 2003). The E2-mediated down-regulation of E6 and E7 transcription results in the release of the p53 and pRB cellular proteins, and allows the normal differentiation process of the host cell. Then, a putative late promoter activates the capsid genes, L1 and L2. Finally, the viral particles are assembled in the nucleus, and the complete virions are released when the cornified layers of the epithelium are shed. The virions are shed in an environment with desquamated cells in the absence of lysis or necrosis, and this further contributes to virus persitence because it avoids inflammation (Stanley, 2008).

Most women infected with a specific HPV type will not show evidence of that same type after 6-12 months. It is not known whether the HR-HPV can be detected for periods similar to those for LR-HPV. Some studies show similar duration (Richardson *et al*., 2003), but others reveal longer durations of infection for HR-HPV types (Franco *et al*., 1999; Ho *et al*., 1998). It appears that HR-HPV, particularly HPV16, has a longer time to clearance and is more likely to develop persistent infection (Richardson *et al*., 2003).

2.4.3 Role of CD4+ T lymphocytes in Disease Progression

The CD4+ T lymphocytes; a subpopulation of the lymphocytes also known as T helper cells, are coordinators of the body's immune response, e.g., providing help to B cells in the production of antibody, as well as in augmenting cellular immune response to antigens. The “CD” or cluster of differentiation is a protein expressed on the surface of the cells of the hematopoetic system. The expression of these proteins is used in lymphocyte nomenclature. Over 300 CD molecules have

been reported so far. These proteins are often associated with the specific function of the cells. Cells with different functions express different CD molecules (for eg: CD3+ cells are total T lymphocytes, CD4++ cells are T helper cells, CD8+ cells are cytotoxic T lymphocytes and CD19+ are B lymphocytes). CD4+ T lymphocytes occupy the central position in regulating immune functions. CD4+ T lymphocytes are the primary targets of HIV. The relentless destruction of CD4+ T lymphocytes by HIV, either directly or indirectly, results in the loss of HIV-specific immune response, recall antibody response and, finally, non-specific immune response in the AIDS stage (WHO, 2007).

2.4.4 Immune Response in HPV and HIV Infection

Human papillomavirus (HPV) infections have been reported to be prevalent in human immunodeficiency virus (HIV) positive individuals (Dolei *et al.*, 1999). There is evidence to show that HIV-positive women have a high prevalence of HPV infections in the cervix (Heard *et al.*, 2000; Palefsky, 2006; Sun *et al.*, 1995) and a study conducted by Ahdieh and colleagues (2001) showed that HIV-positive women were 1.8, 2.1 and 2.7 times more likely to harbor high-, intermediate-, and low-risk HPV infections, respectively, than HIV-negative women. The persistence of HPV lesions was approximately twice greater in women with a CD4 cell count less than 200 cells/μl compared with greater than 500 cells/μl (Ahdieh *et al.*, 2001).

The number of sexual partners is one of the key risk factors for HPV acquisition. This is in agreement with findings that have suggested that individuals infected with HIV tend to have a higher prevalence of anogenital infections, (Critchlow *et al.*, 1992; Palefsky, 2006) with a lower CD4+ count being one of the most consistent risk factors for anogenital intraepithelial neoplasia.

Thus, immune suppression due to HIV infection may play an important role in the development of high-grade intraepithelial neoplasia and eventual progression to cancer (Palefsky, 2006). The importance of cell-mediated immunity in the control of HPV infection has been evidenced by studies that have documented an increased prevalence and progression of HPV infections in the immunosuppressed (Scott *et al.*, 2001; Stanley, 2006). Multiple recurrences of cervical HPV infections occur in HIV infected patients (Fruchter *et al.*, 1996; Stanley, 2006). Human Immunodeficiency Virus may attenuate the systemic immune response against HPV via its effect on CD4+ cells and regulation of immune responses to different types of antigens. A low number of circulating HPV specific memory cells is thought to make the HPV-specific immunity vulnerable to the effects of HIV (Palefsky, 2006).

2.5 Clinical features of Human papillomavirus

The clinical features of Human papillomavirus has been sumarised in the Table 2.1.

The early stages of cervical cancer caused by HPV may be completely asymptomatic (Kumar *et al.*, 2007). Virginal bleeding, bleeding or rarely a virginal mass often dirty bloody and purulent discharge may indicate the presence of malignancy. Also, moderate pain during sexual intercourse and discharge are symptoms of cervical cancer. In advanced disease, metastases may be present in the abdomen, lungs or elsewhere (Canivan and Doshi, 2000). Symptoms of advanced cervical cancer may include; loss of appetite, weight loss, fatigue, pelvic pain, back pain , leg pain, single swollen leg, heavy bleeding from the vagina, leaking urine or faeces from the vagina and bone fractures (Nanda, 2006)

Table 2.1 Human Papillomavirus Types and Clinical Manifestations

Clinical manifestations	HPV Types
1. Common warts	2, 7
2. Plantar warts	1, 2, 4, 63
3. Flat warts	3, 10, 8
4. Anogenital warts	6, 11, 42, 44 and others (11)
5. Anal lesions	6, 16, 18, 31, 53, 58
6. Genital cancers	Highest risk: 16, 18, 31, 45 Other high-risk: 33, 35, 39, 51, 52, 56, 58, 59 Probably high-risk: (12) 26, 53, 66, 68, 73,82
7. Epidermodysplasia verruciformis	more than 15 types
8. Focal epithelial hyperplasia (oral)	13, 32
9. Oral papillomas	6, 7, 11, 16, 32
10. Oropharyngeal cancer	16
11. Verrucous cyst	60
12. Laryngeal papillomatosis	6,11

Source: Gomez and Santos (2007).

2.6 Diagnosis

Diagnosis is the signs and symptoms that an individual acquire due to the presence of an infectious organism in the body. Human papillomavirus can be detected using the following diagnostic techniques:

2.6.1 Colposcopy

Colposcopy is a technique used for the evaluation of a abnormal cervical cytology smear. The colposcope is an instrument that uses illuminated low power magnification (5-15x) to inspect the cervix, vagina, vulva, or anal epithelium. Abnormalities in the appearance of the epithelium and its capillary blood supply often are not visible to the naked eye but can be identified by colposcopy, particularly after the application of 3-5% acetic acid solution (Cristine and Holschneider, 2003). The main goal of colposcopy is to prevent cervical cancer by detecting precancerous lesions early and treating those infected (Chase *et al.*, 2009).

2.5.1 Signs and Symptoms

2.6.2 Serology

The serological detection of antibodies against HPV antigens is a useful tool to determine exposure to genital HPV infection and in predicting the risk of persistence infection and associated lesions (Wang *et al.*, 2004). Enzyme-linked immunosorbent assays (ELISAs) are commonly used for seroepidemiological studies of HPV infection but are not staderdized. Specific ELISA for detecting IgG, IgM, and IgA antibody to HPV are available (Petter *et al.*, 2000).

Serological testing for circulating anti-HPV antibodies has potential clinical utility in measuring cumulative exposure to HPV infection and has a marker of HPV-associated disease, particularly for the anogenital tract (Palefsky, 1995). Long-term persistent HPV infection tends to yield polyclonal immunoglobulin G (IgG) serological reactivity, which can be measured using ELISA techniques based on type specific L1-only or L2-plus-L2 virus-like particles (VLPs) as antigens (Petter *et al.,* 2000). Serological testing for HPV antibodies cannot replace direct HPV DNA measurements or cytological examinations of the target tissue, but it can serve as an adjuvant tool, particularly in molecular epidemiology studies of the natural history of HPV infection and cervical precancerous lesions (Carter *et al.,* 2000; Wang *et al.,* 2004). Thus far, however, HPV serology has not been used in the clinical setting because of a variety of technical and biological limitations (Palfesky, 1995; Nonnenmacher *et al.,* 2003). Although the specificity of HPV ELISA is reasonably adequate, at over 90%., its sensitivity is relatively low (50% - 60%) (Tabrizi *et al.,* 2006).

2.6.3 Cervical cytology smear (Papanicolaou test)

Papanicolaoun test also called the Pap test was first developed by G. N. Papanicolaou and his colleagues in the 1940s. A Pap test can detect pre-cancerous and cancerous cells in the vagina and cervix. The cervix is the lower part of the uterus that connects the vagina with the main part of the uterus.

In carrying out the Pap test, a small sample of cells would be removed from the cervix with a brush or spatula. The procedure is not painful as only the surface of the cervix is touched. The cells would then be smeared onto a slide and examined under a microscope. A Pap test can

identify abnormal cells and detect changes before cancer has fully developed (Behtash and Mehrdad, 2006; Vince and Lepej, 2010).

The direct smear method has been used for a long time, and is relatively inexpensive. Cell samples are placed directly on a glass slide and sent to the lab. New developments have made the test more efficient, but they are significantly more expensive. It has not been determined yet if the new methods increase detection sensitivity enough to warrant the increased cost. The two new methods are liquid-based cytology, in which the cell sample would be fixed with a special liquid before being placed on the slide to make the cervical cells more visible and automated detection of abnormal cells utilizing a machine (Vince and Lepej, 2010).

2.6.4 Molecular diagnosis methods for human papillomavirus

The molecular method of detection of HPV is by the detection of HPV DNA through molecular biology techniques, regardless of the method used, which or is based on the specificity of the complementary between the nucleic acids (Alba *et al.,* 2009). A DNA sequence has the ability to hybridize with other DNAs or RNAs so specifically that at a certain temperature it only forms hybrids if 100% of the bases are complementary. After sample collection (biopsy), samples are extracted and lysis is requiring releasing the viral DNA from the samples. A variety of commercial DNA extraction reagents are available. Hence, molecular methods aimed at detecting HPV always target the L1, upstream regulatory region (URR) or the E6/E7 genes which are always retained in infected and neoplastic cells (WHO, 2009).

Hybridization solution: hybrid capture: this method uses RNA probes that have the capacity to hybridize with the viral DNA in solution and be detected by luminescent methods. The modern commercial methods, such as the so-called Hybrid Capture II, unlike the previous

versions that were regarded as sub-optional, have an adequate relationship between sensitivity and specificity if limits of luminous signal are imposed. The use of a cocktail of high risk probes, that in the latest version includes 13 types and another for the group of low-risk, which includes 5 types, allows the detection of these viral types. It has the advantage of the possibility to semi-quantify the viral load, although this quantification only indicates number of viral copies and cannot be corrected as a function of the number of cells obtained in the samples. The main disadvantage is that it is neither able to distinguish between different viral types nor the presence of multiple infections and several studies cite non-specificities due to cross-reaction between the high risk probes nor certain low risk viral types (Kang *et al.,* 2009).

Polymerase chain reaction (PCR) method: like the hybrid capture, PCR uses small DNA probes that traces specific sequences of viral DNA and are called primers. The fundamental difference with the other techniques is in the chain amplification in the region of interest, which can then be visualized by different techniques such as ELISA, electrophoresis, laser detection (Alba *et al.,* 2009).

There are two fundamental alternatives for detection of HPV based on conventional PCR (de Araujo *et al.,* 2009). Firstly and as the most frequently used method, we count on PCR of consensus regions, where a region with sequences, which are very similar among all the HPVs, is amplified, and then by methods of specific hybridization, enzymatic or of DNA sequencing, make specific typing of the virus possible. The most popular consensus method uses the common region L1, using MY09 and MY11 "primers" (Baseman and Koutsky, 2005). The amplified region generated is approximately 450 pairs of bases but has certain disadvantages, such as not allowing the detection of type 35. For this reason, new "primers" such as PGMY09/11, were

synthesized with a relative increase in sensitivity and spectrum of viral detection, and others such as GP5/GP6 or its improved variant GP5+/GP6+ (Jacobs *et al.,* 1997; de Araujo *et al.,* 2009).

Specific PCR is based on the design of "primers" for specific viral types, subtypes or variants and allows us to adjust the conditions of the reaction of femtogram sensibilities, with specificity around 100 per cent. Moreover, it allows the realization of analysis of viral integrational genes (Alba *et al.,* 2009).

DNA sequencing i.e, the obtaining of the sequence of nucleotides that shapes a particular region of viral DNA, is undoubtedly the reference standard for any of the previous techniques. Though this method can amplify or clone any fragment of viral DNA and determine their nucleotide composition and compare the result with the database of all the known sequences of HPVs to determine to what type, subtype or variant the sample belongs. Its main drawback is the cost and the need for sophisticated laboratories to be able to use this methodology. The advantage, apart from giving direct and unequivocal typing is to distinguish viral variants and polymorphisms, which are currently being considered as highly risk variables in neoplastic transformation (Alba *et al.,* 2009).

2.7 Treatment of Human Papillomavirus

There is no treatment for Human papillomvirus. It can only be prevented or controlled.

2.8 Prevention and Control of Human Papillomavirus

Human papillomavirus infection is the most frequent sexually transmitted disease in the world. Methods of prevention include abstinence from sex, use of condoms, vaccination and use of microbicides (Gavillon *et al.,* 2010).

2.8.1 Condoms (Diaphram)

The Centers for Disease Control and Prevention (US) says that male "condom use may reduce the risk for genital human papillomavirus (HPV) infection" but provides a lesser degree of protection compared with other sexual transmitted diseases "because HPV also may be transmitted by exposure to areas (e.g., infected skin or mucosal surfaces) that are not covered or protected by the condom (CDC, 2009). Female condoms provide somewhat greater protection than male condoms, as the female condom allows for less skin contact (Toronto, 2011). Studies have suggested that regular condom use can effectively limit the ongoing persistence and spread of HPV to additional genital sites in individuals already infected (Moscicki, 2005 and Bleeker *et al.,* 2005).

2.8.2 Human papillomavirus vaccine

Two vaccines are available to prevent infection by some HPV types: Gardasil, marketed by Merck, and Cervarix, marketed by GlaxoSmithKline. Both protect against initial infection with HPV types 16 and 18, which cause most of the HPV associated cancer cases. Gardasil also protects against HPV types 6 and 11, which cause 90% of genital warts (Gavillon *et al.,* 2010). The vaccines provide little benefit to women having already been infected with HPV types 16 and 18, which include most sexually active females (WHO, 2009). For this reason, the vaccine is recommended primarily for those women not yet having been exposed to HPV during sex. The World Health Organization position paper on HPV vaccination clearly outlines appropriate, cost-effective strategies for using HPV vaccine in public sector programs (WHO, 2009).

Both vaccines are delivered in three shots over six months. In most countries, they are approved only for female use, but are approved for male use in countries like USA and UK. The vaccine

does not have any therapeutic effect on existing HPV infections or cervical lesions (Markowitz *et al.,* 2007). In 2010, 49% of teenage girls in the US got the HPV vaccine, while in comparison around two-thirds of teens received shots for meningitis and Diphtheria Pertussis and Tetanus toxoids (DPT) vaccine (Teen Vaccination against Cervical Cancer Lagging, 2011).

Women should continue to seek cervical screening, such as Pap smear testing, even after receiving the vaccine. Cervical cancer screening recommendations have not changed for females who receive HPV vaccine (Markowitz *et al.,* (2007). With or without continued screening, the number of cervical cancers preventable by vaccination alone is less than the number of cervical cancers prevented by regular screening alone (Harper, 2009 and Harper, 2010). Both men and women are carriers of HPV. The Gardasil vaccine also protects men against anal cancers and warts and genital warts (Gavillon *et al.,* 2010).

2.8.3 Adverse effect of HPV vaccine

The most common adverse reactions reported during clinical trials of HPV vaccines were local reactions at the site of injection (CDC, 2007). In pre-licensure clinical trials, local reactions, such as pain, redness or swelling were reported by 20-90% of recipients (CDC, 2007). A temperature of 0.47° C during the 15 days after vaccination was reported in 10-13% of recipients of either vaccine. A similar proportion of placebo recipients reported an elevated temperature. Local reactions generally increased in frequency with increasing doses. However, reports of fever did not increase significantly with increasing doses. No serious adverse events have been associated with either HPV vaccine based on monitoring by CDC and the Food and Drug Administration (CDC, 2010). A variety of systemic adverse reactions were reported by vaccine recipients,

including nausea, dizziness, myalgia and malaise. However, these symptoms occurred with equal frequency among both vaccine and placebo recipients (CDC, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Barau Dikko Teaching Hospital, Kaduna in Kaduna State. Kaduna state is in Northern Nigeria. Kaduna state population in 2016 was approximately 1,582,102. It occupies an area of 46,053km ^{2} and is located at coordinates: 10.52 ^{o} N 7.44^{o} E (http://www.maps-streetview.com/.../kaduna/).

3.2 Study Population

The study was conducted among HIV positive women who came for their routing check-up's and antiretroviral drug collection at the Barau Dikko Teaching Hospital, Kaduna in Kaduna State.

3.3 Inclusion Criteria and Exclusion Criteria

3.3.1 Inclusion criteria;

All HIV positive women who came for their regular CD4+ count and gave their consent were enrolled for the research.

3.3.2 Exclusion criteria;

All HIV positive women who refused to give their consent, women who were HIV negative, children and men.

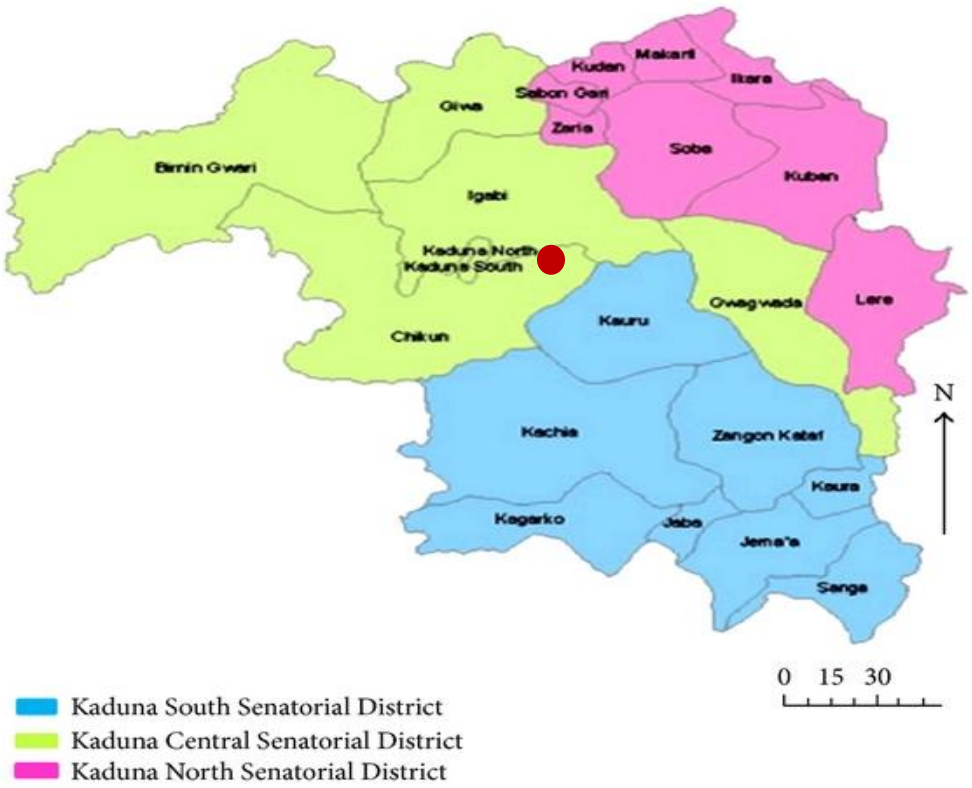


Figure 3.0 Map indicating the study area. The shaded circle on the map represents the study area.

Adapted from: Nigerian Muse, (2010).

3.4 Study Design

The study design was a descriptive cross-sectional and experimental study combining the use of administrative structured questionnaire and analysis of serum samples taken from consented patients.

3.5 Ethical Considerations

Ethical consent was obtained from the Ministry of Health, Kaduna state and the chief matron of Barau Dikko Teaching Hospital, Kaduna. Informed consent was also obtained from the patients to be enrolled before commencement of the study (Appendix I and II).

3.6 Sample Size Determination

The sample size was determined using the following equation as described by (Naing *et al.*, 2006).

$$n = \frac{z^2 p (1-p)}{d^2}$$

where n= sample size

z= statistics for a level of 95% confidence interval =1.96

p= prevalence rate at 11.7% Kano Nigeria (Auwal *et al.*, 2012).

d= precision (allowable error) = 5% = 0.05

Therefore; $n = \frac{(1.96)^2 \times 0.117 \times (1- 0.117)}{(0.05)^2}$

=158.75

=159 samples

Attrition rate = 10% of 159 sample size

= 174.9

= 175 samples.

The calculated sample size was 175 which was the least number of samples to be used. Therefore, 270 women were enrolled in the study.

3.7 Collection of Samples

After counseling and recruitment, blood samples were collected with the assistance of a Laboratory Scientist from patients who gave consent. A total of 270 blood samples were collected from 270 HIV positive women who were enrolled in the study. All materials required for collection of the samples were assembled and each vacutainer was labeled with patient's (subject) identification (ID) and date. For the blood collection, a venipuncture was made with needle, the vacutainer was fixed and 5ml of blood was collected. The blood samples was allowed to clot for 30min and centrifuged at 1000xg for 10min to separate the serum. The serum of each patient was carefully removed with a transfer pipette in order to avoid extracting red cells and transferred aseptically into a sterile labeled serum storage screw-capped container and was stored in the freezer set at -20^{o}C until analysed.

3.8 Data Collection

Prior to sample collection, a structured questionnaire was administered to the HIV positive women to be analysed to obtain their socio-demographic data and clinical information related to HPV infection (Appendix III).

3.9 Becton Dickinson FACS Count Microbead-based System.

FACS Count system is the product of Becton Dickinson (BD) Biosciences. It is the only available microbead-based single-platform instrument that is designed specifically for enumerating the absolute CD4++, CD8+ and CD3+ T-cell counts in no-lyse, no-wash whole blood (WHO, 2007). This system requires ready-to-use, twin-tube reagent tubes: One tube determines the absolute number of helper/inducer T-cells (CD4+/CD3) or CD4++ T-cells by using a combination of two-colour monoclonal anti-human CD3 antibody conjugated to the tandem dye phycoerythrin +Cy5 (PECy5) and a monoclonal anti-human CD4+ antibody conjugated to phycoerythrin (PE). The other tube determines the absolute number of Cytotoxic/suppressor Tcells (CD8-PECy5/CD3-PE) or CD8+ T-cells. Both tubes also give the absolute number of total T-cells (CD3) or CD3+ T cells, as well as CD4+/CD8 ratio. In addition to the antibody reagents, the reagent tubes also contain a known number of fluorochrome-labeled reference beads. These beads function as fluorescence standard for locating the lymphocytes and also as a quantitation standard for enumerating the cells (WHO, 2007).

The control set consists of fluorescent beads at four different densities: zero (0 beads/µl), low (50 beads/µl), medium (250 beads/µl), and high (1000 beads/µl). When whole blood is added to the reagents, fluorochrome-labeled antibodies in the reagents bind specifically to lymphocyte surface antigens. After a fixative solution is added, the sample is run on the instrument. Here the

stained cells come in contact with the green HeNe laser light, which causes the cell to fluoresce. This fluorescent light provides the information necessary for the instrument to count the cells. The calculation of absolute CD3+, CD4++ and CD8+ T-cells is determined automatically by using the built-in Attractors software program. The system is robust and requires minimal training and hence can be used in a laboratory where the sample load is <50 samples /day. The system can provide absolute CD4+ counts and for obtaining the percentage of CD4+ cells, the ALC from the Haematology analyzer has to be used (WHO, 2007).

3.9.1 Assay for CD4+ count using BD FACS count

Materials: Reagents are BD FACS Count reagent and BD FACS Count control. Supplies are 50µl pipette tips, EDTA vacutainer tube, FACS flow, FACS Rinse, FACS Clean and Bleach. Equipments are Vortex Mixer, Coring Station, Electronic Pipette, and BD FACS Count Machine (Cytometer). Sample control is whole blood of known HIV negative person. Quality control are CD4+ low, CD4+ medium and CD4+ High (Becton Dickinson FACS Count System User's Guide).

3.9.2 Assay for patient's CD4+ count

Three reagent tubes were labeled as CD4+ low, CD4+ medium and CD4+ High. Each tube was vortexed upside down for 5 seconds and then vortexed upright for 5 seconds. The reagent tubes were then opened with the coring station. The sample control was mixed adequately by inverting the tube. Fifty microliter (50µl) of normal Whole blood was pipette into each tube. The tubes were caped and vortexed upright for 5 seconds after which they where incubated for 60-120 minutes at temperature of 20-25^{o}C in the dark. The tubes were uncapped and 50µl of fixative

solution was pipette into each tube. The tubes were recapped and vortexed upright for few seconds. The Low control bead,Medium control bead and the High control bead were placed in the control area of the workstation and then uncapped. The Low control bead was vortexed and and 50µl of Low control bead was was pipette into the CD4 reagent tube labeled Low. The Medium control bead was vortexed and 50µl of Medium control bead was pipette into the CD4 reagent tube labeled Medium. The High control bead was vortexed and and 50µl of High control bead was pipette into the CD4 reagent tube labeled High. The test was Run on the BD FACS Count instrument within 2hrs of adding the control beads (Becton Dickinson FACS Count System User's Guide).

Running patient samples for CD4+ count:

The reagent tubes were vortexed upright for 5seconds. The CD4+ tube was uncapped and the tube was placed in the Sample holder. The "RUN" command was pressed and the CD4+ tube was removed and recapped before disposing off the CD4+ tubes in the bioharzard waste. Patient results were displayed on the screen and are printed automatically. Patient result with CD4+ count between 500-1200cells/mm^3 were regarded as normal CD4+ lymphocyte count while those with CD4+ count less than 200 cells/mm^3 were regarded as low CD4+ lymphocyte count and is one of the indication that an individual is approaching the AIDS stage of the HIV infection (Becton Dickinson FACS Count System User's Guide).

3.10 Screening of Human Papillomavirus Immunoglobulin G (IgG) Antibody using Enzyme Linked Immunosorbent Assay (ELISA)

3.10.1 Principle of the assay

Virus-like particles (VLPs) or self-assembled HPV L1 protein capsids that resemble intact virions are used as antigen to coat the microtitre plate wells. These VLPs present type-specific conformational epitopes are the key reagent in determining assay sensitivity and specificity. Human serums are allowed to react with the antigen. If HPV type-specific antibodies are present, they will bind to the VLPs absorbed onto the surface of the well. Bound human immunoglobulin G (IgG) is detected with a secondary antibody conjugated to horseradish peroxidase (HRP) conjugate reagent. The antigen-antibody- enzyme-antigen complex is stop with sulphuric acid solution. A colorimetric substrate allows wells with HPV IgG antibody-positive samples to be identified by reading the optical density.

3.10.2 Reagent preparation

The wash buffer concentrate was brought to room temperature and mixed gently until completely dissolved. A 30fold of the wash buffer concentrate was poured into a measuring cylinder an distilled water was added to it to reach 600ml of wash buffer solution.

3.10.3 Assay for HPV ELISA

All reagents were brought to room temperature and mixed thoroughly by gentle swirling before used. Samples were numbered according to the microlitter well. Fifty (50µl) of the positive and negative control were added to the positive and negative well. Forty (40µl) of the sample diluent was added into appropriate well, followed by 10µl of the sample sera to the bottom of the coated well and was mixed gently. For the blank well 40µl of the sample diluent was added. The plates

was covered with a plate membrane cover and incubated for 30min at 37^{o}C. After the period of incubation, the plate membrane cover was removed and the liquid discarded. The plate was dried by gentle swinging, then washing buffer was added to each well and allowed to stand for 30sec, after which it was drained and dried by blotting using tissue. The washing was repeated 5 times. Fifty (50µl) of HPR-conjugate was added to each well except blank well and incubated for 30 min at 37^{o}C. The liquid was discarded, dried by swirling then washing buffer was added to each well and allowed to stand for 30sec then drained and dried by blot drying. Fifty (50µl) of chromogen solution A and chromogen solution B was dispensed into each well and incubated for 15min at 37^{o}C, then 50µl of stop solution was added to each well to stop the reaction. When the color changed from blue to yellow within 15min, the plates were read at an absorbance of 450nm using a microplate reader (WKEA Medical Supplies, Changchun, China).

3.10.4 Interpretation of result

The calculation of cut off value was done using the manufacturer's specifications. The average of negative control well plus 0.15 was given as the cut off value. Negative HPV IgG antibody results were interpreted as any sample whose optical density was less than the calculated cut off value and samples with optical density greater than the calculated cut off value were reported as HPV positive.

3.11 Data Analysis

Results from the test analysis and data from questionnaires were reduced to percentages and presented on tables. Statistical analysis was done using SPSS version 17 software programme. Associations and relationships between variables were determined by Spearman's rho correlation and P values ≤ 0.05 were considered significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Analysis of the Results

Two hundred and seventy samples were collected from HIV positive women attending Barau Dikko Teaching Hospital in Kaduna, Nigeria and Human papillomavirus (HPV) IgG antibody was detected in 21.9% (59/270) of the women. Result on Table 4.1 shows the analysis on the prevalence rate of HPV in HIV positive women and indicate that 59(21.9%) of the HIV positive women attending Barau Dikko Teachng Hospital had their HPV status to be positive while 211(78.1%) of the HIV positive women had their HPV status as negative.

The result analysed based on age group showed that the women in the age group of 20-29 years old had the highest prevalence of 38.9%(21/54) for HPV and the least prevalence (2.9%: 1/34) was recorded in the age group >60 years old (Table 4.2). Infection rate with HPV differed significantly with age of the women (P=0.035, X^2=10.335, df=4).

The prevalence of HPV analysed according to the demographics of the studied population (Table 4.3). Women belonging to the Hausa tribe had the highest HPV prevalence of 30.4% (24/79) while the lowest prevalence was observed in the Yoruba and Igbo language who had 14.3% (2/14) and 14.3% (3/21) respectively. The difference in tribe of the women was not statistically significant (P=0.160, X^2=5.165, df=3). The analysis of the result has shown that out of 121 Muslims that were analysed, 34 of them were positive for HPV 28.1(34/121). The least prevalence 16.8(25/149) of HPV was observed in the Christians. However, the HPV result reveals a statistically significant association between the infection and religion. Analysis of the

result based on their occupation has shown that HPV was most prevalent among the Civil servant 30.4% (17/56) and least prevalent among the retired 0% (0/1) although the result was not statistically significant (P=0.272, X^2=3.903, df=0.272).

Result on Educational status indicate that HPV was most prevalent among those HIV positive women with no education 42.9% (6/14) and the result was significant (P=0.279, X^2=5.080, df=4). The entire population of women analysed claimed to be non smokers and had HPV prevalence of 100% (59/270) and as a result, had no computable statistical analysis.

Based on the different forms of contraceptives used by the women analyzed, HPV was most prevalent 25.9% (36/139) among those that did not use any form of contraceptive and least prevalent 16.0% (8/50) among those women that used Pills as contraceptive though the result was not statistically significant (P=0.395, X^2=4.082, df=4).

The distribution of HPV (Table 4.4) shows the distribution of HPV according to family style among the HIV positive women being analysed and their marital status indicated that HPV had the highest prevalence 29.3 (12/41) among the singles while divorced women had the least prevalence (0.4%: 1/12). There was no significant difference between HPV infection and their marital status. Highest prevalence (22.9%:39/170) was observed among women married into monogamous homes and least prevalent (2.6%:11/39) observed among the unmarried although the difference observed was not statistically significant (P=0.445, X^2=2.672, df=3).

Table 4.1: Prevalence of HPV IgG Antibody in HIV Positive Pomen

HPV Status	Frequency	Percentage (%)
Positive	59	21.9
Negative	211	78.1
Total	**270**	**100.0**

(P=0.025, X^2=5.011,df=1).

Table 4.2: Prevalence of HPV according to Age Group of HIV Positive Women attending Barau Dikko Teaching Hospital in Kaduna Metropolis, Nigeria

Age group (years)	Number Analysed	Number Positive	Percentage (%)	P-value	Chi square value (X^2)	Degree of freedom (df)
				0.005	10.335	4
20-29	54	21	38.9			
30-39	127	29	22.8			
40-49	45	6	13.3			
50-59	10	2	20.0			
60-69	34	1	2.9			
TOTAL	**270**	**59**				

P<0.05 [Chi Square (χ^2)] = Significant

Table 4.3: Prevalence of HPV according to Demographic Characteristics among HIV Positive Women attending Barau Dikko Teaching Hospital in Kaduna Metropolis, Nigeria

Variable	Number Analysed	Number Positive	Percentage (%)	P value	Chi square value(X^2)	Degree of freedom(df)
Tribe				0.160	5.165	3
C	79	24	30.4			
D	14	2	14.3			
E	21	3	14.3			
Others	156	30	19.2			
Religion				0.025	5.011	1
A	121	34	28.1			
B	149	25	16.8			
Occupation				0.272	3.903	3
Unemployed	129	23	17.8			
Self employed	84	19	22.6			
Civil servant	56	17	30.4			
Retired	1	0	0			
Education				0.79	5.082	4
Primary	53	8	15.1			
Secondary	102	23	22.5			
Tertiary	77	17	22.1			
Qur'anic	24	5	20.8			
None	14	6	42.9			
Smoking						
Yes	0	0	0			
No	270	59	21.9			
Use of contraceptives				0.395	4.082	4
Oral	5	1	20.0			
Injection	45	11	24.4			
Inserted Plastic	31	3	9.7			
Pills	50	8	16.0			
None	139	36	25.9			

$P<0.05$ [Chi Square (χ^2)] = Significant

Key: A= Islam, B= Christianity, C= Hausa, D= Yoruba, E= Igbo

The distribution of HPV (Table 4.4) based on the years of the correspondents in marriage, women who had no years in marriage had the highest HPV prevalence of 33.3% (12/36) and the least prevalence (12.5%: 1/8) among those women who had greater than 40 (>40) years in marriage but the difference was not statistically significant (P=0.752, X^2=0.569, df=2). The HIV positive women who claimed to have never been pregnant before had the highest HPV prevalence of 41% (13/31) and least prevalence (16.7%:9/54) was observed in who had pregnancies between the range of 7-9. The result was not statistically significant (P=0.754, X^2=1.903, df=4). Out of the 47 correspondents that had no children, 16 of them had HPV infection 34.0% (16/47) while out of the 223 women who had children, 43 were HPV positive and the result was statistically significant (P=0.026, X^2=4.952, df=1). Women who had no children had the highest prevalence of 34.0% (16/47) while those that had number of children between (1-3) had the least prevalence of HPV 16.1% (20/123) but the difference in the result was not statistically different (P=0.131, X^2=5.624, df=3).

Table 4.5 shows the distribution of HPV according to history of sexual activity and genetic disorder among HIV positive women and the table shows that 50% (1/2) of the women who claimed not to have had any sexual contact before had HPV infection while those that had their First Sexual Intercourse (FSI) before 9yrs and greater than thirty years (>30) had the least prevalence of 0%(0/3) and 0%(0/9) respectively. The result was not statistically significant (P=0.170, X^2=6.418, df=4). Based on the age at First Child Birth (FCB), HPV prevalence was highest (33.3%:14/42) among women who had never given birth and least (19.4%:20/103) among women who were between the age group 20-29. The result was statistically significant (P=0.100, X^2 =6.261, df=3).

Table 4.4: Distribution of HPV according to Family Style among HIV Positive Women attending Barau Dikko Teaching Hospital in Kaduna Metropolis, Nigeria

Family Style	Number Analysed	Number Positive	Percentage (%)	P value	Chi square value(X^2)	Degree of freedom(df)
Marital status				0.445	2.672	3
Single	41	12	29.3			
Married	178	38	21.4			
Widow	39	8	2.1			
Divorce	12	1	0.4			
Type of marriage				0.242	2.840	2
Monogamy	170	39	22.9			
Polygamy	61	9	14.8			
None	39	11	2.6			
Years in marriage				0.752	0.569	2
1-9	63	17	27.0			
10-19	113	17	15.0			
20-29	38	9	23.7			
30-39	12	3	25.0			
40-45	8	1	12.5			
None	36	12	33.3			
Parity				0.754	1.903	4
0-3	84	15	17.9			
4-6	95	20	2.2			
7-9	54	9	16.7			
9-12	6	2	33.3			
None	31	13	41.9			
Children				0.026	4.952	1
Yes	223	43	19.3			
No	47	16	34.0			
No. of Children				0.131	5.624	3
1-3	123	20	16.1			
4-6	84	20	23.5			
7-12	16	3	18.8			
None	47	16	34.0			

$P<0.05$ [Chi Square (χ^2)] = Significant

Table 4.5: Distribution of HPV according to History of Sexual Activity and Disorder among HIV Positive Women attending Barau Dikko Teaching Hospital in Kaduna Metropolis, Nigeria

Variable	Number Analysed	Number Positive	Percentage (%)	P value	Chi square value (X^2)	Degree of freedom(df)
Age of FSI						
8-9	3	0	0	0.170	6.418	4
10-19	135	31	23.0			
20-29	121	27	22.3			
30-34	9	0	0			
None	2	1	50			
Age at FCB				0.100	6.261	3
10-19	118	23	19.5			
20-29	103	20	19.4			
30-34	7	2	28.6			
None	42	14	33.3			
Abnormal Discharge				0.115	2.481	1
Yes	82	13	15.9			
No	188	46	24.5			
Virginal Complaint (general complains)				0.112	2.519	1
Yes	46	6	13.0			
No	224	53	23.7			
History of CC				0.642	0.216	1
Yes	15	4	26.7			
No	255	55	21.6			
History of GD				0.969	0.002	1
Yes	14	3	21.4			
No	256	56	21.9			

$P<0.05$ [Chi Square (χ^2)] = Significant

Key: FSI= First Sexual Intercourse, FCB= First Child Birth, CC= Cervical cancer, GD= Genetic Disorder

Thirteen women out of the 82 women (15.9%:13/82) who had abnormal discharge had HPV infection while 24.5% (46/188) of women who had no abnormal discharge were HPV positive though the result was not statistically significant (P=0.115,X^2=2.481, df=1). Out of the 46 women that had vaginal complaints, 13.0% (6/46) had HPV infection while 23.7% (53/224) of the women who had no vaginal complaints were HPV positive. The result was not statistically significant (P=0.112, X^2=2.519, df=1).

Table 4.5 also had shown that, out of the 15 women that had history of cervical cancer, 4 0f them (26.7%) had HPV infection while 55 out of the 255 (21.6%) women that had a history of cervical cancer were HPV positive. However, the result was not statistically significant (P=0.642, X^2=0.216, df=1).

History of Genetic Disorder (GD) (Warts hypogammaglobulinemia, immunodeficiency and myelokathexis) as shown on the Table 4.5 indicate that 21.4% (3/14) of the women who had the history had HPV antibodies in their serum while 21.9% (56/256) of women who did not have the history of Genetic disorder were HPV positive and the result was not statistically significant (P=0.969, X^2=0.002, df=1). Result on Prevalence of HPV according to CD4+ count of the HIV positive women indicate that HIV positive women who had their CD4+ count less than 200cells/ mm^3 and were positive for HPV were 6 out of 28 (21.4%) while those HIV positive women who were positive for HPV and had their CD4+ count greater than 200cells/ mm^3 were 53 out of 241 (22.0%) and had the highest prevalence. The result was non significant (P=0.670, X^2=0.181, df=1).

Table 4.6 tabulates the spearman's rank order correlation on HPV status and CD4+ count. The result of the analysis indicated that there was no statistically significant relationship between HPV status and CD4+ count ($r = -0.112$, $p>0.05$).

Table 4.6: Correlation between CD4+ Count and HPV Status

		HPV Status	CD4+ Count
HPV Status	Correlation Coefficient	1.000	-112
	Significance (2-tailed)		0.67
CD4+ Count	Correlation Coefficient	-112	1.000
	Significance (2-tailed)	0.67	

Spearman's rho Correlation ($r = -0.112$, $p>0.05$). No correlation between HPV status and CD4+ count since the 'r' value is negative.

CHAPTER FIVE

5.0 DISCUSSION

This study reports the prevalence of Human papillomavirus IgG antibody among HIV positive women attending Barau Dikko Teaching Hospital in Kaduna, Nigeria. Human papillomavirus antibody was detected in 21.9% (59/270) of the HIV positive women been analysed. The prevalence obtained in this study was similar to that of Ezechi *et al.* (2014) who reported an overall hr HPV prevalence of 19.6% and also 18.3% reported from Ibadan, Nigeria (Thomas *et al.,* 2004), but the prevalence is higher than 14.6% reported from Irun, a rural and agrarian community in Osun state of Nigeria (Gage *et al.*, 2012), suggesting a possible lower prevalence of hr HPV infection in rural communities. This finding is also confirmed by previous research in low-income countries, including Nigeria, that the prevalence of sexually transmitted infection is higher in urban than in rural areas (Piras *et al.*, 2011; Ebisi, 2012). This may be related to varying sexual behaviour, lifetime sexual partnership and practices in the two settings.

The mean CD4+ count of the women was 492 cells/ mm^3 which indicate that most of the women in the study population had adequate immune cell population. This may be the reason why CD4+ count and HPV status of the women had no correlation. This study had also shown that the prevalence of HPV among women with CD4+ count >200cells/ mm^3 was higher (22.0%:53/241) compared to 21.4% (6/28) prevalence obtained among those that had their CD4+ count less than (<) 200cells/ mm^3. This is in contrast with the results of several studies that have consistently shown a higher prevalence of HPV infection and greater persistence of HPV infections in HIV positive women with CD4+ count < 200 cells/ mm^3 (Palefsky *et al.*, 1999; Strickler, *et al.*, 2005; Ngandwe *et al.,* 2007). This could be as a result of the HIV positive women receiving Anti-

Retroviral Treatment (ART) which help to boost their immunity but would not prevent them from having HPV infection since both HPV and HIV are mostly sexually transmitted diseases and can be contacted irrespective of an individual's CD4+ count.

There were a number of socio-demographic factors that were predictive of high risk HPV status in the HIV positive population. The highest prevalence of HPV in this study was found in the age group 20-29 years and followed by the age group 30-39 years. The least prevalence was found in women greater than 60 years old. This finding is similar to what was discovered among HIV positive Rwandan women in whom prevalence peaked in those aged 25-34years and declined in those greater than 55 years old (Singh *et al.,* 2009). This may be explained by the early indulgence in sexual activities and early marriage (Christine and Holschneider, 2003). It is known that 70% of HPV infection disappears in 1 year and 90% in 2 years (Goldstein *et al.,* 2009).

The highest prevalence of HPV in this study was recorded among the civil servants. This could be due to the hardship in acquiring jobs where women engage themselves in sexual activities in other to acquire job, sustain or gain promotions in their work places.

Although all the respondents in this study claimed to be non smokers, other studies have shown that local immune suppression induced by smoking and the mutagenic activity of cigarette components have been demonstrated in cervical cells, and may contribute to persistence of HPV or to malignant transformation similar to that seen in the lung (Yang *et al.,* 1996).

In this study, women who do not use contraceptives had the highest prevalence of HPV which is in agreement with the studies of (Marrazzo *et al.,* 2001; Naucler *et al.,* 2007), that recorded a higher prevalence of the infection among women who did not use any form of contraceptives. This could be due to unprotected sex which is the major route of transmitting HPV infection (Clifford *et al,* 2005).

It has been shown that the level of education as a socioeconomic factor has an effect on HPV infection. The people with no education had the highest prevalence in this study which was in support with the work of Schlecht *et al.* (2001) and Naucler *et al.* (2007) which showed that HPV infection rate reduces with increase in level of education. The reason for the high prevalence among women with no education could be due to their early indulgence in sexual activities, lack of awareness about the infection and its mode of transmission.

Based on marital status, the single women had the highest prevalence of HPV infection in this study which is similar to the findings of Tabora *et al.* (2005) and Sellors *et al.* (2003). This finding is in contrast to the findings of Menacndez *et al.* (2010) and Mesmoudi *et al.* (2011) who observed a higher prevalence among married women. This difference in findings could be due to similar rate of sexual activities among the single and married women and the singles involving them selves in multiple sexual partners.

This study reports a higher prevalence of HPV among women married into monogamous homes than polygamous homes. This is in contrast to the general belief that sexual activities among different partners expose them more to the infection then a single partner. This could be due to the monogamous husbands not being faithful to their wife and getting involved with multiple partners and as a result infecting their wife.

The number of years in marriage had no significant effect on HPV infection, implying that HPV can be acquired at any time of marriage. Similarly, parity had no significant effect on HPV infection as the highest prevalence of the infection was recorded among those women who claim to have never been pregnant. The observation in this study is in contrast to the findings of Schlecht *et al.* (2001) and Okolo *et al.* (2010). This is also in contrast to the report of American Cancer Society (ASC) in 2012 that, women who have had multiple pregnancies have an increased risk of developing cervical cancer due to having unprotected intercourse to get pregnant. This is in contrast to the findings in this study and reasons could be far fetched as ladies who claim to have never been pregnant could be in sexual relationships which may or may not have resulted in pregnancies that they choose not to report.

Women who had no children had higher prevalence of HPV which was statistically significant (P=0.026) than those who had children. This is in contrast to the finding of Firnhaber *et al.* (2010). This could be as a result of trying to conceive from other partners order than their husbands especially if they had spent many years in marriage without conceiving in the case of married women while the singles could have had several sexual partners and series of abortions in order not to get pregnant.

Based on the age of first sexual intercourse, women who claimed to have never had sexual intercourse had the highest of 50% (1/2), followed by those that had their first sexual intercourse between the age of 10-19 years. The high prevalence among the young women is in agreement with the findings of Naucler *et al.* (2007) and Okolo *et al.* (2010) who reported increase of HPV among young women due to indulgence in sexual activities at early age. The strong association between sexual behaviour and HPV infection observed in younger females in this study could be

due to early exposure to sexual activities rather than transient or latent infections in non immunocompromised hosts.

In this study, women who had no children had the highest prevalence of HPV infection. This is in contrast with the finding of ASC, (2012) who reported that women who were younger than 17 years when they had their first pregnancy were almost 2 times more likely to get cervical cancer later in later in life than women who waited to get pregnant until they were 25 years or older. The reason for the women with no children having the highest prevalence could be due to the singles not having desire for children and still engaging in sexual activities with multiple partners. The married women who have no children could be tempted to have multiple partner also due to their desperation to have children.

Abnormal discharge was found in 15.9% (13/82) of the women in this study though the result was not statistically significant. Abnormal discharge in HPV is always seen when neoplasia set in and in advance stage of the HPV infection (Christine and Holschneider, 2003). Vaginal complaints like itching, rashes and ulcers were found among 13% (6/46) of the women been infected with HPV in this study. These signs and symptoms have always been associated with Cervical Intraepithelial Neoplasm (CIN) and cervical cancer and known to be associated with painful intercourse (Pitts and Clarke, 2002). Since the number of people with vaginal complains were low, their symptoms might not necessarily be an indication of having cervical cancer.

Four out of 15 (26.7%) of the women with history of cervical cancer in their families had HPV infection. American Cancer Society (2012) reported that, if in a family cervical cancer is known to be genetic, the chances of developing the disease is 2 to 3 times higher than if no one in the family has it. Some researchers suspect that some instances of this familial tendency were caused

by an inherited condition that makes some women less able to fight off HPV infection than others (ACS, 2012).

History of genetic disorder Warts hypogammaglobulinemia, immunodeficiency and myelokathexis (WHIM) was reported among 21.4% (3/14) of those women whom their family members had genetic disorder and where positive to HPV infection. Warts hypogammaglobulinemia, immunodeficiency and myelokathexis (WHIM syndrome) is a rare congenital immunodeficiency disorder characterized by chronic noncyclic neutropenia (Qian *et al.*, 2011). It is known to be associated with extensive infection with HPV and repeated bacterial infection (Qian *et al.*, 2011).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Human papillomavirus IgG antibody was detected in 21.9% of the HIV positive women attending Barau Dikko Teaching Hospital in Kaduna metropolis, Nigeria.

The virus was detected with highest prevalence among the age group 20-29 years old and the association was statistically significant. This indicates that these women may likely develop cervical cancer if the infection persists for years. Other risk factors associated with the virus in this study were religion, educational status, having children and the age at first sexual intercourse.

The virus was also detected with high prevalence among women who do not use contraceptives and have a family history of cervical cancer.

There was no correlation between HPV status of the women and their CD4+ count, and as such, their association was statistically non-significant.

6.2 Recommendation

1. Since there is high incidence of HPV infection especially among HIV positive individuals in Nigeria and other developing countries, there is the need to educate people on the prevalence of HPV in the population in order to avoid unnecessary morbidity and mortality.

2. The importance of routine HPV screening should be emphasized in order to avoid the persistence of the infection and development of cervical cancer due to ignorance.

3. Government should provide the populace with adequate screening centers and provide services at subsidized rates.

4. Vaccines against wide range of high-risk types HPV should be made available and administered to teenagers and adults prior to their engagement in sexual activities. This would help to reduce the risk of acquiring other oncogenic HPV types capable of progressing to cervical cancer as against the available ones that were produced to prevent HPV types 16 and 18 only.

5. Public enlightenment should be made available to educate people on the risk factors associated with HPV and how to help women make responsible choices regarding their sexual practices.

6. Further studies on the prevalence of high risk types of HPV among HIV positive and HIV negative women is recommended. This study would help in determining the choice of vaccines to be produced for use in a particular geographical area.

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APPENDIX 1

APPENDIX 2. Table 4.1: Distribution of the respondents by socio-demographics

Variable (%)	Frequency	Percentage
Age group (years)		
20-29	68	25.2
30-39	134	49.6
40-49	43	15.9
50-59	14	5.2
60-62	11	4.1
Marital status		
Single	41	15.2
Married	178	65.9
Widow	39	14.4
Divorce	12	4.4
Occupation		
Unemployed	129	47.8
Self employed	84	31.1
Civil servant	56	20.7
Retired	1	0.4
Educational Qualification		
None	14	5.2
Primary	53	19.6
Qur'anic	24	8.9
Secondary	102	37.8
Tertiary	77	28.5
Religion		
A	121	44.8
B	149	55.2
Use of contraceptives		
Oral	5	1.8
Injection	45	16.6
Inserted Plastic (Diaphragm)	31	11.5
Pills	50	18.5
None	139	51.5
Tribe		
C	79	29.3
D	14	5.2
E	21	7.8
Others	156	57.8
Smoking		
Yes	0	0
No	270	100

Key: A= Islam, B= Christianity, C= Hausa, D= Yoruba, E= Igbo

Table 4.2: Distribution of women by reproductive characteristics and sexual behaviour

Variable	Frequency	Percentage (%)
Type of marriage		
Monogamy	170	63.0
Polygamy	61	22.6
NA	39	14.4
Years in marriage		
1-10	63	23.3
10-19	113	42.6
20-29	38	14.1
30-39	12	5.6
40-43	8	3.0
None	36	13.3
Parity		
0-3	84	31.1
4-6	95	35.2
7-9	54	20.0
10-12	6	2.2
None	31	11.5
Children		
Yes	223	82.6
No	47	17.4
No. of Children		
1-3	123	45.5
4-6	84	31.1
7-12	16	5.9
None	47	17.4
Age of first sexual intercourse		
8-9	3	1.1
10-19	135	50.0
20-29	121	44.8
30-34	9	3.3
None	2	0.7
Age at first child birth		
10-19	118	43.7
20-29	103	38.1
30-34	7	2.6
None	42	15.6

Table 4.3: Distribution of the study population based on their CD4+ count

CD4+ Count Range (cell/mm^3)	Frequency	Percentage (%)
<200	28	10.4
200-399	77	28.5
400-599	85	31.5
600-799	39	14.4
800-999	30	11.1
1000-1199	9	3.3
1200>	2	0.8
Total	**270**	**100**

Mean CD4+ count: 492 cell/ mm^3.

(>) Means greater than.

(<) means less than

APPENDIX 3: INFORM CONSENT

RESEARCH TOPIC: Prevalence of Human Papillomavirus Among HIV positive women Attending Barau Dikko Specialist Hospital, Kaduna, Kaduna State.

I am MSc. Microbiology student with Ahmad Bello University Zaria. I intend to carry out my research on the topic stated above.

I am interested in knowing the prevalence of Human papillomavirus (HPV) because it has been reported that HPV is the necessary factor for the development of cervical cancer. Cervical cancer has been ranked the second most common cancer in females and it is also the leading cause of cancer deaths among women in Nigeria and in the developing countries.

Complications in immunocompromised HIV patients due to HPV and other sexually transmitted diseases (STIs) are a major cause of mother and child mortality and morbidity during pregnancy.

Due to limited information on the prevalence of HPV in Africa, the incidence of HPV in causing cervical cancer are likely to increase in areas where there is limited awareness of the infection and where there is no routine screening for the virus.

I would like to advice the Government based on my findings to create more awareness on HPV infection as it is the leading cause of cervical cancer, provide vaccines against the common HPV types that would be detected and also advice the general populace on how the infection can be prevented.

In view of this, I would like to obtain your consent to take your blood samples and cervical scrapings for the research topic mentioned above. You have the right to accept or reject this request. Also at any point in time of this research that you have change of mind, you are free to withdraw. Thank you.

Participant initials, signature/date Researcher initials, signature/date.

Witness initials, signature/date

APPENDIX 4 :STRUCTURED QUESTIONAIRE

RESEARCH TOPIC: Prevalence of Human Papillomavirus Among HIV Positive women attending Barrau Dikko Specialist Hospital, Kaduna, Kaduna state.

Kindly assist by filling the appropriate information as all information will be treated and kept confidential.

SECTION A: BIODATA

1. Serial No……..... Hospital……
2. Religion…….....
3. Tribe……...
4. Age 15-19[ ] 20-24 [ ] 25-34 [ ] 30-34[ ] 35-39 [ ] 40-44[ ] 45-49 [ ] 50-54[ ] 55-60[ ]
5. Occupation: Civil servant [ ] Self employed[ ] Unemployed[ ] Retired[ ] Others[ ]
6. Highest educational level: Primary[ ] Secondary[ ] Tertiary[ ] Quranic[ ] None[ ]
7. Marital status: Single[ ] Married [ ] Widow[ ] Divorce[ ]

SECTION B: EPIDEMIOLOGICAL RISK FACTORS

8. Any abnormal discharge from the vagina? Yes [ ] No [ ]
9. Is there itching [ ] Rashes[ ] Ulcer[ ] None [ ]
10. Any family history of cervical cancer? Yes[ ] No[ ]
11. Any family history or genetic disorder? Yes[ ] No[ ]
12. How old were you when you had your first sexual intercourse?
13. What type of marriage are you in? Monogamy [ ] Polygamy[ ] Polyandry [ ]
14. How long have you been married?.....
15. How many pregnancies have you had?.....
16. Do you have children? Yes[ ] No[ ]
17. How many children do you have? ……..
18. At what age did you had your first child?.....
19. What type of contraceptive do you use: oral[ ] injection[ ] Pills[ ] Inserted plastics[ ] Others[ ]
20. Do you smoke? Yes[ ] No[ ].

APPENDIX 5: OD results of ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
	OD	0.095	0.000	0.115	3.024	2.757	0.090	0.112	0.172	0.093	0.000	0.148	0.083
B	Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
	OD	0.113	0.085	0.173	0.000	4.000	0.002	0.123	0.132	0.072	0.119	0.151	2.299
C	Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
	OD	0.055	0.000	0.044	1.060	0.287	0.046	0.129	2.525	0.296	0.082	0.631	0.032
D	Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
	OD	0.088	0.163	0.203	0.068	0.037	0.000	0.000	0.012	0.015	0.005	0.031	0.057
E	Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
	OD	0.050	0.000	1.264	0.245	0.027	0.006	0.599	0.991	0.072	0.000	0.069	2.426
F	Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
	OD	0.241	0.000	0.199	0.000	0.021	0.093	0.000	0.055	0.000	0.001	0.030	0.082
G	Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
	OD	0.020	0.000	0.000	0.000	0.000	1.252	2.014	0.000	0.369	0.000	0.009	0.051
H	Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
	OD	0.075	0.047	0.504	0.231	0.069	0.000	0.000	0.191	0.390	0.052	0.117	1.260

Plate 2

A	Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11
	OD	0.073	0.000	0.002	2.609	2.767	0.030	0.012	0.102	0.081	0.577	0.048	0.052
B	Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
	OD	0.022	0.020	0.060	0.029	0.170	0.575	0.000	0.022	0.057	0.039	0.041	0.118
C	Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35
	OD	0.242	0.000	0.000	0.025	0.025	0.038	0.295	0.021	0.073	0.000	0.000	0.045
D	Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
	OD	0.018	0.013	0.039	0.000	0.090	0.000	0.000	0.001	0.015	0.032	0.019	0.000

E	Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
	OD	0.067	0.000	0.204	0.004	0.476	0.035	0.043	0.000	0.060	2.061	0.199	0.054
F	Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
	OD	0.042	0.017	0.052	0.018	0.083	0.000	0.830	0.118	0.085	0.011	0.017	0.004
G	Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
	OD	0.619	0.000	0.000	0.076	0.058	0.030	0.052	0.156	0.110	0.000	0.015	0.108
H	Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
	OD	0.053	0.028	0.094	0.058	0.073	0.418	0.061	0.033	0.023	0.012	0.029	0.002

Plate 3

A	Hole	B	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
S11												
	OD	0.012	0.011	0.000	0.619	0.475	0.090	0.000	0.904	0.072	0.179	0.341	0.003
B	Hole	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23
	OD	0.075	0.047	0.504	0.231	0.069	0.000	2.001	0.191	0.390	0.052	0.017	1.260
C	Hole	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	
S35													
	OD	0.050	0.000	1.264	0.245	0.027	0.006	0.599	0.991	0.072	0.703	0.069	2.426
D	Hole	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47
	OD	0.055	0.000	0.044	1.060	0.287	0.046	0.129	2.525	0.296	0.082	0.631	0.032
E	Hole	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59
	OD	0.053	0.028	0.094	0.058	0.073	0.418	0.061	0.033	0.023	0.012	0.029	0.002
F	Hole	S60	S61	S62	S63	S64	S65	S66	S67	S68	S69	S70	S71
	OD	0.042	0.017	0.052	0.018	0.083	0.000	0.830	0.118	0.085	0.011	0.017	0.004
G	Hole	S72	S73	S74	S75	S76	S77	S78	S79	S80	S81	S82	S83
	OD	0.018	0.013	0.039	0.000	0.090	1.065	0.000	0.001	0.015	0.032	0.019	0.000
H	Hole	S84	S85	S86	S87	S88	S89	S90	S91	S92	S93	S94	S95
	OD	0.018	0.013	0.039	0.000	0.090	0.000	0.478	0.001	0.015	-	-	-