PREVALENCE AND MOLECULAR CHARACTERISATION OF HUMAN PAPILLOMA VIRUS AMONG WOMEN ATTENDING SELECTED HOSPITALS IN FEDERAL CAPITAL TERRITORY, ABUJA, NIGERIA

(A PhD Research)

By

AONDONA, Priscilla Yahemba

(PhD/SLS/2015/777)

DEPARTMENT OF MICROBIOLOGY,

SCHOOL OF LIFE SCIENCES,

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGERIA

JULY, 2021

ABSTRACT

Cervical Cancer is the second most common cancer caused by Human Papilloma Virus (HPV).Human papillomavirus infection is the most common sexually transmitted viral infection globally, with approximately 75% of sexually active women and men contracting an infection at some point during their lives.HPV infection and cervical cancer have been associated for a long time. HPV is discovered in 99.7% of cervical cancer specimens. This study determined the prevalence and molecular characterisation of HPV infection among women attending selected hospitals in Federal Capital Territory, Nigeria. A total of five hundred and one (501) women volunteers were screened using Enzyme Linked Immunosorbent Assay (ELISA). HPV DNA was obtained by extraction and HPV types identified by PCR method using consensus primer sets MY09/MY11 and $GP5^{+}/GP6^{+}$. Phylogenetic analysis was determined using CLUSTALW on UniproUgene software. Data from administered questionnaires was analysed using Chi square (χ^2) at 95% confidence interval and 0.05 significance level. The analysis was performed using IBM Statistical Package for Social Sciences version 23 statistical software package. The overall prevalence of HPV infection in this studywas 10.98%. Women who participated were within the ages of 15 to 64 years. The results obtained showed high prevalence of HPV among women within the age range of 35 – 44 years (4.80%), while a low prevalence was observed among women between the age range of 15 – 24 years (0.20%).Married women were found to be more infected (7.98%) when compared to the women that were single, divorced or widowed. In consideration of occupation, there was a high prevalence among civil servants (5.60%) compared to farmers and other occupations. Women with tertiary education qualification recorded more rate of infection (7.90%) when compared to those with lower level of education. Demographic data and risk factors such as Age group (χ^2= 9.508, P = 0.050), Educational Status (χ^2 = 55.909, P = 0.000), Marital Status (χ^2 = 15.390, P = 0.000), HIV Status (χ^2 = 11.871, P = 0.001) and Number of sexual Partners (χ^2 = 6.252, P = 0.012) were found to have significant association with HPV infection. Nine HPV types (HPV- 6, 16, 18, 31, 58, 66, 70, 72 and 81) were detected in this study with HPV- 70 being the most predominant (26.67%).The phylogenetic tree was constructed using 15 isolates from this study against 45 reference strains selected from NCBI data based on percentage similarity. HPV-6 isolated in this study clustered with isolate from Iran while HPV-18 clustered with isolate from Bayelsa, Nigeria. Mutation was also detected in HPV 6 and HPV 18 strain isolated in the study. There is a need to increase the level of surveillance on females at risk of cervical cancer in the study area, since significant proportion of highly oncogenic strains with a high tendency to transform into malignancy were observed in this study. There is the need for sexual behaviour education and awareness about HPV to be intensified in order to reduce the spread of the infection in the study area.

TABLE OF CONTENTS

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background to the study

Human Papilloma Virus (HPV) is an icosahedral virus, measuring 55nm in diameter. The viruses are non-envelope, double stranded DNA measuring about 8 kilo bases in length (Brooks *et al.,* 2007). The virus belongs to the Papillomaviridae family and is capable of infecting humans. Like all Papilloma viruses, HPV establishes productive infections only at the stratified epithelium of the skin or mucous membrane leading to the development of cancer (Brooks *et al.,* 2007). Human papillomavirus (HPV) infection is the most common sexually transmitted viral infection globally, with approximately 75% of sexually active women and men contracting an infection at some point during their lives (zur Hausen, 2009).HPV infection and cervical cancer have been associated for a long time; HPV is discovered in 99.7% of cervical cancer specimens (Walboomers *et al.,* 1999; Denny *et al.,* 2014).

Approximately 200 species of HPV have been identified and their classification was dependent on the degree of homology between the viral genomes detected by DNA hybridization (WHO/ICO, 2014). About 40 HPV species have been identified to be associated with the genital mucosal, which are categorized according to their carcinogenic potential (WHO/ICO, 2014). The virus is mostly transmitted through sexual intercourse and it is not easily detected at the early stage of infection. Clinical manifestations of HPV infection include genital warts, recurrent respiratory papillomatosis, Cervical Intraepithelial Neoplasia (CIN), and cancers, including cervical, anal, vaginal, vulva, penile, head and neck cancer (Avci *et al.,* 2013).

Cervical cancer is the second most common malignant cancer in women and is a major cause of cancer related death among women worldwide (Anorlu *et al.,*2007; Fadahunsi *et al.,* 2013; Kolawole *et al.,* 2015). Studies have shown that about 500,000 new cases are diagnosed every year with approximately 85% of deaths occurring in developing countries of the world (Ferlay *et al.,* 2010; WHO, 2012). The incidence of cervical cancer is 14,550 per 100,000 and the mortality rate is 9,659 per 100,000 (WHO, globocan2012*).*

Epidemiological studies have shown that human papillomavirus (HPV) is the main cause of cervical cancer and precancerous lesions (Walboomers *et al.,* 1999; WHO, 2012). The strains involved in the onset of cancers are known as High-risk HPV (HR-HPV) and they include: HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59 -66 and -68 (Gheit & Tommasino, 2011).

According to global data on HPV infection, Africa has the greatest prevalence (22.1%) (de Sanjose *et al.,* 2007).The prevalence of HPV infection has been reported in Sub-Saharan Africa and it is dependent on the age of the patient and the presence of cytological abnormalities. In some populations, cross-sectional studies have revealed high prevalence rate of infection to be between 20% - 40% among sexually active young women and the prevalence decrease as they age (Brooks *et al.,* 2007).Young age, early age at first sexual contact, sexual promiscuity, and immunosuppression are some risk factors that have all been linked to HPV infection in women. The risk rises as the number of recent and lifetime sexual partners increases. Other factors, such as long-term hormonal contraception use, tobacco smoking, low socioeconomic position, and poor diet, have been linked to HPV infection less consistently (Das *et al.,* 2000; CDC, 2013).

Many molecular methods for HPV testing and typing are currently available but PCR amplification of HPV genomes is the most sensitive technique and can detect between 5 and 100 DNA molecules in a specimen (van den Brule *et al.,* 1993). PCR utilizing consensus

primers directed at relatively conserved regions of the HPV genome allows for the amplification of a broad spectrum of HPV genotypes in a single reaction. A number of different primer combinations amplifying fragments from various regions of the HPV genome have been developed (van den Brule *et al.,* 1993).

The most frequently used amplification systems for the detection of HPV DNA in clinical samples are based on MY09/MY11 and GP5+/GP6+ consensus primer sets mediated PCR which amplifies DNA fragments in the conserved L1 region of approximately 450bp and 150bp respectively (Shikova *et al.,* 2009).

Several studies have point to the fact that any single method or technique for the detecting of HPV may underestimate the true prevalence of HPV in cervical samples and so more techniques should be employed to allow for comparison (Karlsen *et al.,* 1996). Based on these, detection of HR-HPV infections which is crucial for identifying women at increased risk of developing cervical lesion involve the optimization of HPV testing methods. In order to optimize the testing methods, for HPV identification, different primer sets are compared to determine their potency in detecting and flanking the gene of interest (Shikova *et al.,* 2009).

Cervarix and Gardasil are the only two prophylactic HPV vaccines that have been approved by the US Food and Drug Administration (FDA) so far. These vaccinations are available for early prevention against infection with HPV strains that cause cancer. All three HPV vaccines have been shown to protect against HPV types 16 and 18. Furthermore, Gardasil 9 can protect against cervical cancer in up to 90% of cases(Zhai & Tumban, 2016).HPV vaccines have been shown to be both safe and effective, providing long-term protection from HPV infections (Mejilla*et al.,* 2017). To the best of our knowledge, the majority of Nigerian healthcare facilities have yet to implement in their national vaccination program, the HPV vaccine.As a result, statistics on HPV genotypes, regional distribution, and risk factors among

childbearing women are needed. The best HPV vaccines are necessary for the prevention of cervical cancer

1.2 Statement of the Research Problem

Cervical Intraepithelial Neoplasia (CIN); and cancers, including cervical, anal, vaginal, vulva, penile, head and neck cancer are caused by HPV (Malloy *et al.*, 2000). Cervical cancer is the third most common cancer among women and the seventh most common cancer in the world with 250,000 deaths and 500,000 new patients worldwide, annually (WHO, 2012). In Nigeria, the number of women at risk of cervical cancer is estimated to be 50.3million and the number of deaths attributed to cervical cancer is 8,240 (Bruni *et al.*, 2010).

The use of chemotherapy for the treatment of cancer related issues has failed. One option that appears to be promising is the prophylactic measure (use of vaccine) towards effective prevention and control of cancers in Nigeria. This study intends to identify the strains of Human Papilloma Virus (HPV) in circulation within the Federal Capital Territory Abuja, Nigeria in order to enrich the data bank for the formulation of indigenous vaccine for HPV in future.

1.3 Justification for the Study

Previous studies on Human papilloma virus among Nigerian women have been conducted but limited (Thomas *et al.*,2004; Okolo *et al.*,2010; Nnodu *et al.*,2010; Akarolo- Anthony*et al.*,2014; Kolawole *et al.*,2015; Okwuraiwe *et al.*,2015 and Nyengidiki *et al.*,2016). Despite the high rate of cervical cancer morbidity and death in Nigeria, no reliable nationwide data on HPV genotypes in Nigerian women exists. A study by Akarolo- Anthony*et al.* (2014) in Abuja was restricted to just National hospital which is located in one municipal area council as against the six municipal area councils. This study intends to capture all the six municipal area councils and determine the HPV genotypes in circulation within the Federal Capital

Territory, Abuja. This study differs from the previous studies in the sense that aside DNA sequencing of the HPV isolated was performed, phylogenetic analysis was also conducted to know the evolutionary history of the HPV in circulation in Abuja. This study has provided baseline information on the circulating genotypes/subtypes of the HPV in the study area for the development of indigenous vaccine in future.

1.4 Aim and Objectives

Aim

This study determined the prevalence and molecular characterization of HPV among women attending six selected hospitals in the Federal Capital Territory, Abuja, Nigeria.

Objectives

i. To detect HPV from samples obtained from women attending six selected hospitals in the Federal Capital Territory, Abuja, using ELISA technique.

ii. To identify socio demographic and risk factors associated with HPV infection in the study area.

iii. To extract the DNA of HPV from the samples collected and amplify, using PCR technique.

iv. To sequence the amplicons.

v. To construct a phylogenetic tree that will determine the evolutionary relationship of the different strains identified.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HUMAN PAPILLOMA VIRUS

Sexually transmitted HPV types fall into two categories: Low-risk HPV, which do not cause cancer but can, cause skin warts (technically known as C*ondylomata acuminata*) on or around the genitals, anus, mouth, or throat. For example, HPV types 6 and 11 cause 90 percent of all genital warts. HPV types 6 and 11 also cause recurrent respiratory papillomatosis, a less common disease in which benign tumours grow in the air passages leading to lungs. High-risk HPV, responsible for cancers have been identified to include types 16 and 18 (Lowy and Schiller, 2012).

About 14 million new genital HPV infections occur each year (Satterwhite*et al.,* 2013). Center for Disease Control and Prevention (CDC) has estimated that more than 90 percent of sexually active men and 80 percent of sexually activewomen are infected with at least one type of HPV at some point in their lives (CDC, 2012; Chesson *et al.,* 2014).

2.1.1 HPV genome

Human Papilloma Virus a double stranded DNA virus consists of icosahedral capsid composed of 72 capsomers that have shapes resembling five pointed stars. HPV genome exists inside the capsid, and harbours eight partially overlapping open reading frames. The genome is divided into three regions: an early region (E), late region (L), and a long control region (LCR) (Figure 2.1) (Stanley, 2010).

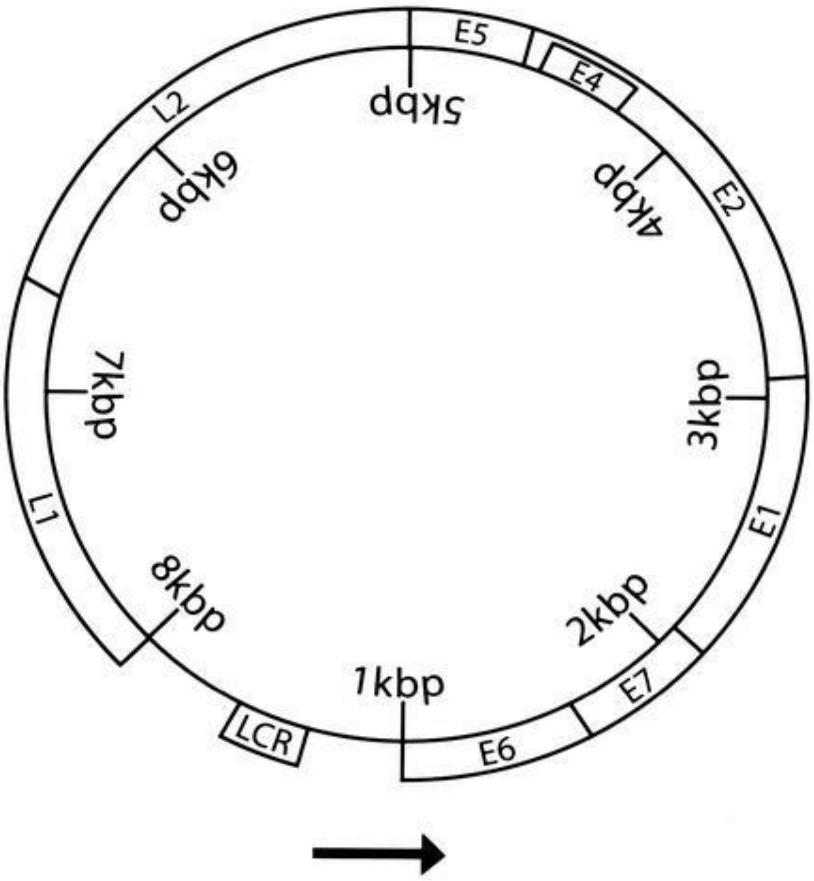


Figure 2.1: Human Papilloma Virus genome

Source: (Burd, 2003)

Table 2.1: Functions of human papillomavirus genes

Region	Gene	Function
Early region (E)	E1	• Enables episomal replication and acts as a replicative helicase
	E2	• Regulates viral transcription; particularly inhibits E6 and E7
	E4	• Binds to cytoskeletal proteins and breaks the cytoskeletal network, contributing to the deformation of infected cells (koilocytosis)
	E5	• Inhibits apoptosis and exposure of histocompatibility complex types I and II; thus preventing a T-cell mediated response
		• Interacts with growth factor receptors
	E6	• Binds to tumour suppressor gene, *p53*
	E7	• Binds to tumour suppressor gene, retinoblastoma (*Rb*)
Late region (L)	L1	• Binds to tumour suppressor gene, *p53*
	L2	• Encodes a major structural capsid protein (55 kDa in size)
		• Encodes a minor structural capsid protein (70 kDa in size)
Long control region	LCR	• Involved in viral replication and transcription

Source: Choi and Park (2016).

2.1.2 HPV classification

The classification of HPV is clinically significant for the following reasons: (1) only one specific HPV genus is associated with cervical cancer; (2) the pathogenicity of HPV varies according to genotype. HPV is grouped into five genera (alpha, beta, gamma, mu, and nu), and the genus *Alphapapillomavirus* includes HPV genotypes that infect both genital and oral mucosa (Stanley, 2010). In addition, HPV can be classified based on its L1 genome sequence type, intra-typic lineage, and sub-lineage. Different types, intra-typic lineages, and sub-lineages of HPV are defined by having L1 sequences that differ by at least 10%, more than 1%, and less than 1%, respectively (Bernard *et al.*, 2010).

2.1.3 Life cycle of HPV

The transformation of HPV infected cells to cancer cells is a multi-step process (Bosch *et al.*, 2008). HPV infects basal cells located in the epithelial transformation zone. This transformation zone exists between the stratified squamous epithelium of the ectocervix and the columnar epithelium of the endocervix, and provides an entry site for HPV. The viral replication process begins shortly after the virus enters a host cell. Initial viral replication is tightly linked to the epithelial cell differentiation cycle. HPV infects only dividing basal epithelial cells; thus, HPV DNA replicates only when basal cell DNA is replicated. Genes E1 and E2 are required for the maintenance of viral genomes in host cells, as they serve as the initial sites for replication of viral DNA, and also recruit cellular DNA polymerase needed for replication. The E6 and E7 oncoproteins act to enhance cellular proliferation, resulting in increased numbers of infected cells and infectious virions (Hamid *et al.*, 2009). In summary, carcinogenesis is a multi-step process, not only because viral genes take various actions to transform a normal cervical cell into a cervical cancer cell, but also because cervical epithelial tissue progresses through phases of being normal epithelium, cervical

intraepithelial neoplasia tissue (CIN; CIN 1, CIN 2, and CIN 3), and carcinoma *in situ*, when developing into cervical cancer. Over expression of viral genes results in the transformation of HPV infected cells to malignant cells (Doorbar, 2005).

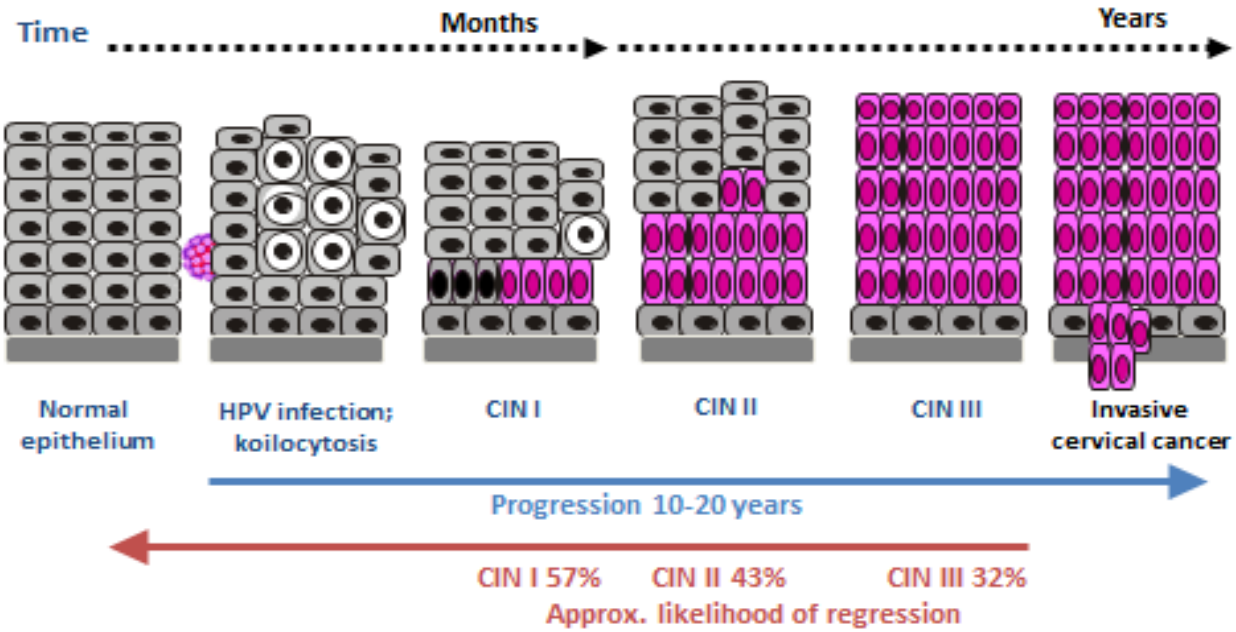


Figure 2.2: Pathological Progression in the tissue

Source: (Burd, 2003)

2.2 Pathogenesis

Transmission of HPV occurs primarily by skin-to-skin contact. Basal cells of stratified squamous epithelium may be infected by HPV. Other cells types appear to be relatively resistant. It is assumed that the HPV replication cycle begins with entry of the virus into the cells of the basal layer of the epithelium. It is likely that HPV infection of the basal layer requires mild abrasion or micro trauma of the epidermis. Once inside the host cell, HPV DNA replication progresses to the surface of the epithelium. In the basal layer, viral replication is considered to be non-productive, and the virus establishes itself as a low-copy number episome by using the host DNA replication machinery to synthesize its DNA on average once per cell cycle (Flores and Lambert, 1997).

In the differentiated keratinocytes of the basal layer of the epithelium, the virus switches to a rolling-circle mode of DNA replication, amplifies its DNA to high copy number, synthesizes capsid proteins, and causes viral assembly (Flores *et al.*, 1999).

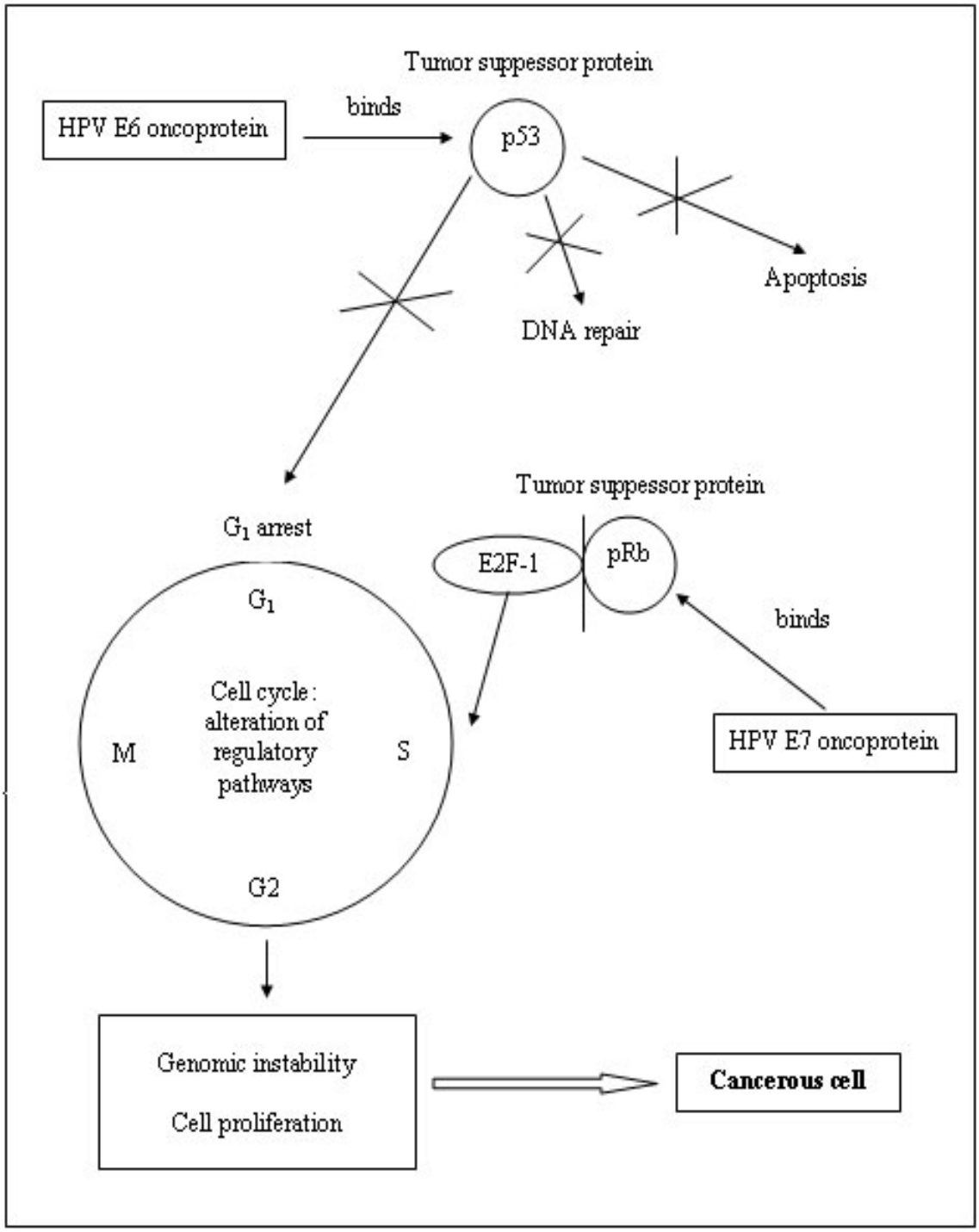


Figure 2.3: Molecular mechanisms of oncogenic HPV infection

Source: Flores *et al*., (1999)

Since HPVs encode only 8 to 10 proteins, they must employ host cell factors to regulate viral transcription and replication. HPV replication begins with host cell factors which interact with the LCR region of the HPV genome and begin transcription of the viral E6 and E7 genes. The E6 and E7 genes products deregulate the host cell growth cycle by binding and inactivating tumour suppressor proteins, cell cyclins, and cyclin-dependent kinases. The function of the E6 and E7 gene products during a productive HPV infection is to subvert the cell growth-regulatory pathways and modify the cellular environment in order to facilitate viral replication in a cell that is terminally differentiated and has exited the cell cycle **(**Syrjanen and Syrjanen, 1999).

Cell growth is regulated largely by two cellular proteins, the tumour suppressor protein, p53, and the retinoblastoma gene product, pRB. Unlike in many other cancers, the p53 in cervical cancer is usually wild type and is not mutated. The HPV E6 gene product binds to p53 and targets it for rapid degradation via a cellular ubiquitin ligase. This degradation has the same effect as an inactivating mutation. As a consequence, the normal activities of p53 which govern G_1 arrest, apoptosis, and DNA repair are abrogated. Low-risk HPV E6 proteins do not bind p53 at detectable levels and have no effect on p53 stability in vitro. The HPV E6 proteins also can form complexes with at least six other cellular proteins which are not well characterized. The HPV E7 gene product binds to the hypo phosphorylated form of the RB family of proteins. This binding disrupts the complex between pRB and the cellular transcription factor E2F-1, resulting in the liberation of E2F-1, which allows the transcription of genes whose products, is required for the cell to enter the S phase of the cell cycle. The E7 gene product can also associate with other mitotically interactive cellular proteins such as cyclin E **(**Syrjanen and Syrjanen,1999).

The outcome is stimulation of cellular DNA synthesis and cell proliferation. The E7 protein from low-risk HPV types binds pRB with decreased affinity. Next, the E5 gene product induces an increase in mitogen-activated protein kinase activity, thereby enhancing cellular responses to growth and differentiation factors. This results in continuous proliferation and delayed differentiation of the host cell (Flores *et al.,* 1999). The E1 and E2 gene products are synthesized next. The E2 gene product is a DNA binding protein which blocks transcription of the E6 and E7 genes and permits the E1 gene product to bind to the viral origin of replication located within the LCR. This binding initiates replication of the viral genome as extra chromosomal elements in the S phase of the cell cycle. Genome copy number is maintained at a constant level in these cells, and a low level of transcripts is expressed (Flores *et al.,* 1999). The E2-mediated down-regulation of E6 and E7 transcription results in the release of the p53 and pRB proteins, and the normal differentiation process of the host cell are allowed to continue. Then, a putative late promoter activates the capsid genes, L1 and L2. Viral particles are assembled in the nucleus, and complete virions are released as the cornified layers of the epithelium are shed. The E4 gene product plays a role in the maturation and release of papillomavirus particles. The process does not appear to be cytolytic. In the replication process, viral DNA becomes established throughout the entire thickness of the epithelium but intact virions are found only in the upper layers of the tissue (Flores *et al.,* 1999).

In warts or condylomata, viral replication is associated with proliferation of all epidermal layers except the basal layer. This leads to acanthosis, parakeratosis, hyperkeratosis, and deepening of rete ridges, creating the typical papillomatous cytoarchitecture seen histologically. Some infected cells transform into koilocytes, which are large, usually polygonal, squamous cells with a shrunken nucleus inside a cytoplasmic vacuole. Excessive

proliferation of cells in the basal layer accompanied by large number of mitoses, some abnormal, is a feature of malignant and premalignant disease (Flores *et al.*, 1999).

Cervical cancer is one of the best understood examples of how viral infection can lead to malignancy. Infection with high-risk HPV types interferes with the function of cell proteins and also with the expression of cellular gene products. Microarray (gene chip) analysis of cells infected with HPV-31 has shown that 178 cellular genes are up-regulated and 150 cellular genes are down-regulated by HPV **(**Chang and Laimins, 2000).

The genes that are down-regulated are primarily those involved in regulation of cell growth, some keratinocyte-specific genes, and interferon (IFN)-responsive genes. High-risk HPV types can be distinguished from other HPV types largely by the structure and function of the E6 and E7 gene products. In benign lesions caused by HPV, viral DNA is located extra chromosomally in the nucleus. In high-grade intraepithelial neoplasia and cancers, HPV DNA is generally integrated into the host genome. In some cases, episomal and integrated HPV DNAs are carried simultaneously in the host cell. Integration of HPV DNA specifically disrupts or deletes the E2 ORF, which results in loss of its expression **(**Yoshinouchi, *et al.*, 1999)**.**

This interferes with the function of E2, which normally down-regulates the transcription of the E6 and E7 genes and leads to an increased expression of E6 and E7. In high-risk HPV types, the E6 and E7 proteins have a high affinity for p53 and pRB. Binding disrupts the normal function of these cellular proteins and can give rise to an increased proliferation rate and genomic instability. As a consequence, the host cell accumulates more and more damaged DNA that cannot be repaired. Efficient immortalization of keratinocytes requires the cooperation of the E6 and E7 gene proteins; however, the E7 gene product alone at high levels can immortalize host cells. Eventually, mutations accumulate that lead to fully

transformed cancerous cells. In addition to the effects of activated oncogenes and chromosome instability, potential mechanisms contributing to transformation include methylation of viral and cellular DNA, telomerase activation, and hormonal and immunogenetic factors. Progression to cancer generally takes place over a period of 10 to 20 years. Some lesions become cancerous more rapidly, sometimes within a year or two **(**Holowaty *et al.*, 1999)**.**

2.3 Epidemiology

Transmission of HPV occurs primarily by skin-to-skin contact. Epidemiologic studies clearly indicate that the risk of contracting genital HPV infection and cervical cancer is influenced by sexual activity. HPV is very resistant to heat and desiccation and nonsexual transmission via fomites can also occur, such as by prolonged exposure to shared contaminated clothing **(**Roden *et al.,* 1997**)**. An individual is at greater risk of becoming infected with HPV if he or she has had multiple sexual partners at any time or is the partner of someone who has had multiple sexual partners. Sexual activity at an early age also places an individual at increased risk, as does a history of other sexually transmitted diseases, genital warts, abnormal Pap smears, or cervical or penile cancer in an individual or sexual partner. Condom usage may not adequately protect individuals from exposure to HPV since HPV can be transmitted by contact with infected labial, scrotal, or anal tissues that are not protected by a condom.In addition to sexual activity, age is an important determinant of risk of HPV infection **(**Adam *et al.*, 2000).

Most cervical cancers arise at the squamocolumnar junction between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix. At this site, there are continuous metaplastic changes. The greatest risk of HPV infection coincides with greatest metaplastic activity. Greatest metaplastic activity occurs at puberty and first pregnancy and

declines after menopause. HPV infection is most common in sexually active young women, 18 to 30 years of age. There is a sharp decrease in prevalence after 30 years of age. However, cervical cancer is more common in women older than 35 years, suggesting infection at a younger age and slow progression to cancer. Persistence of infection is more common with the high-risk oncogenic HPV types and is an important determinant in the development of cervical cancer (Adam *et al.*, 2000).

Detection of high-risk HPV is necessary but may not be sufficient for the development of cervical cancer. Studies suggest that whether a woman will develop cervical cancer depends on a variety of additional factors that act in concert with cancer-associated HPV types in the process that leads to cervical cancer. The primary immune response to HPV infection is cell mediated; therefore, conditions that impair cell-mediated immunity such as renal transplantation or human immunodeficiency virus disease increase the risk of acquisition and progression of HPV **(**Calore *et al.*, 2001**).** The upstream regulatory region of HPV contains sequences similar to the glucocorticoid responsive elements that are inducible by steroid hormones such as progesterone (the active component of oral contraceptives) and dexamethasone. Long-term use of oral contraceptives is a significant risk factor for high-grade cervical disease according to some studies but not in others **(**Adam *et al.*, 2000**).**

Cervical cancer risk also seems to be independently influenced by other variables including current smoking and parity. 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 **(**Cubie *et al.*, 2000**).** It appears that smoking is the most important risk factor independent of HPV infection for higher grades of cervical disease **(**Adam *et al.*, 2000**).** Smoking shows little or no relationship to low grades of cervical disease. Multiple

pregnancies were a significant independent risk factor among women with histopathologic evidence of HPV infection in biopsy specimens and among women with moderate- to high-grade cervical disease. In women with mild cervical disease, only the presence of high-risk HPV infection was a significant risk factor. Other factors such as alcohol consumption and diet have not been well established (Adams *et al.*, 2000).

There has been some suggestion that sexually transmitted viruses may serve as cofactors in the development of cervical cancer. It has been postulated that co-infection with herpes simplex virus type 2 may play a role in the initiation of cervical cancer (zur Hausen, 2009). Cytomegalovirus (CMV), human herpes virus 6 (HHV-6), and HHV-7 have also been detected in the cervix. Co-infection offers the opportunity for these viruses to interact with HPV. Putative oncogenes and transforming factors have been proposed for CMV and HHV-6, but epidemiologic and in vitro data are not conclusive of a causal association with cervical cancer. Recent studies using PCR to detect CMV, HHV-6, and HHV-7 in women with abnormal cervical cytologic test results indicate that these viruses are only bystanders rather than cofactors in the development of cervical cancer **(**Chan *et al.*, 2001**).**

It has been proposed that the viral load correlates directly with the severity of disease. Studies using quantitative type-specific PCR for high-risk HPV-16, -18, -31, -33, and -45 and low-risk HPV-6 and -11 have shown that HPV-16 can reach much higher viral loads than the other types and that only for HPV-16 does increased viral load correlate with increased severity of cervical disease. High-risk HPVs of all types are able to induce malignant tumours even when they are present at low levels **(**zur Hausen, 1996).

An important emerging factor in the development of cervical neoplasia is the role of HPV variants. HPV variants differ in biological and chemical properties and pathogenicity. Based on sequence variation of the L1, L2, and LCR regions of HPV-16, five naturally occurring

phylogenetic clusters have been defined for HPV-16: European (E), Asian (As), Asian-American (AA), African-1 (Af1), and African-2 (Af2). Intratypic sequence variation has also been found in the E2, E4, E5, E6, and E7 genes of HPV-16. Since the LCR contains several E2 binding sites in addition to binding sites for several transcription factors, nucleotide sequence variation in the LCR, E2, E6, and E7 genes may be of functional significance. The oncogenicity of specific HPV variants appears to vary geographically and also with the ethnic origin of the studied. One study suggested that because of increased transcriptional activity population and changes in the progesterone response elements, Asian-American variants might have enhanced oncogenic activity compared to European isolates **(**Veress *et al.*, 1999).

European HPV variants with point mutations in the LCR binding sites have enhanced transcriptional activity compared to the European prototype. In a large clinical study of 10,000 women in Costa Rica, the European HPV-16 prototype and three variants were seen. The most common variant, EP[a], contained a single point mutation and was not associated with disease. A second variant, EL, contained single point mutations at locations other than that of EP[a] and was associated with normal cytology and some high-grade squamous intraepithelial lesions (HSILs). Another variant, NE, contained multiple substitutions within the LCR and was associated with HSIL and cancer at rates much higher than expected. Mechanisms for this association are not known, and since transformation is a complex process, mutations could directly affect transcription by increasing the activity of promoters, could affect other regions of the viral genome, or could affect the relationship between HPV variants and non-viral factors such as HLA and p53. The study also reported a statistically significant association of the NE variant with the presence of HLA class II alleles. Other studies have also reported the association of HLA class II alleles with cervical HPV disease; however, these associations appear to be relatively weak. Intratypic sequence variation has also been analysed for other high- and intermediate-risk HPV types and for low-risk types 6

and 11. Sequence variation in low-risk types was not associated with increased activity of the promoters responsible for E6 and E7 protein expression (Magnusson *et al.*, 2000)**.**

A genetic predisposition to colorectal cancer, lung cancer, and melanoma has long been recognized and is widely accepted. Genetic predisposition was found to be even a greater component in cervical cancer when the same method of analysis was used. Genetic heritability was found to account for 27% of the effect of underlying factors for tumour development. Heritability could affect many factors contributing to the development of cervical cancer, including susceptibility to HPV infection, ability to clear HPV infection, and time to development of disease. The effect of shared familial environment was shown to be small at 2% and was found only between sisters and not between mother and daughter.

Studies have shown that infections with multiple types of HPV can occur **(**Quint *et al.*, 2001**).** Multiple-infection rates up to 39% have been seen. The presence of multiple HPV genotypes tended to increase with the severity of cervical disease. Multiple genotypes, usually with at least one type classified as high risk, were found in 11.8% of patients with normal cytology or ASCUS and in 34.5% of patients with mild or moderate dyskaryosis. However, the prevalence of multiple genotypes was much lower in cervical carcinoma tissue samples (4.4%). The majority of multiple infections contain two HPV genotypes, but samples with three, four, or five genotypes were also seen **(**Quint *et al.*, 2001**).**

2.4 Risk factors

Epidemiologic studies indicate that the risk of contracting genital HPV infection and cervical cancer is influenced by a variety of factors. High-risk HPV infection is necessary but may not be sufficient for the development of cervical cancer. Cervical cancer depends on a variety of additional factors that act in concert with cancer associated HPV types. Cervical cancer is

closely associated with poverty, poor access to health services, rural living, and low educational attainment, with the greatest burden occurring among middle-aged women (WHO, 2005)

2.4.1 Sexual factors

Numerous studies clearly indicate that the risk of contracting genital HPV infection and cervical cancer is influenced by sexual activity. An individual is at greater risk of becoming infected with HPV if he or she had multiple sexual partners at any time or is the partner of someone who has had multiple sexual partners. Sexual activity at any early age also has an increased risk of HPV infections, as does a history of other sexually transmitted diseases, genital wart, abnormal Pap smears or penile cancer in an individual or sexual partner. Condom usage may not adequately protect individuals from exposure to HPV since it can be transmitted by contact with infected tissue that is not protected by a condom.

In addition to sexual activity, age is an important determinant of risk of HPV infection. Most cervical cancer arises at the squamo-columnar junction between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix. At this site, there are continuous metaplastic changes. The greatest risk of HPV infection coincides with greatest metaplastic activity. Greatest metaplastic activity occurs at puberty and first pregnancy and declines after menopause. The HPV prevalence reaches its peak in young adults (18 to 30 years of age) and declines at older ages. However, cervical cancer is common in women older than 35 years, suggesting infection at a younger age and slow progression to cancer (de Villiers, 2001).

2.4.2 Viral factors

Persistent cervical infection (often defined as an infection that is detected more than once in an interval of 6 months or longer) with an oncogenic HPV type (especially HPV-16 and HPV-18) is the most important risk for progression to high-grade dysplasia and invasive cancer. The risk of progression depends on the HPV type. Four to Six years follow-up of 1,643 women with normal cytology showed that women with a positive PCR high-risk HPV DNA test at baseline were 116 times more likely to develop CIN 3 than women with a negative DNA test. The risk of progression for HPV-16 and HPV-18 is greater than for other HPV type, approximately 40%. It has been proposed that the viral load correlate directly with the severity of disease. Studies using quantitative type-specific PCR for high-risk HPV and low-risk HPV have shown that HPV-16 can reach much higher viral loads than the other type, and that only for HPV-16 high viral loads correlate with increased severity of cervical disease. However, high-risk HPV are able to induce malignant tumours even when they are present at low levels (Gomez & Santos, 2007).

An important emerging factor in the development of cervical neoplasia is the role of HPV variants. HPV variants differ in biological, chemical and pathogenic properties. The oncogenicity of specific HPV variants appears to vary geographically and also with the ethnic origin of the population studied. Based on sequence variation of L1, L2 and URR regions of HPV-16, five variants have been defined for HPB-16: European (E), Asian (AS), Asian-American (AA), African-1 (AF1) and African-2 (AF2). Asian-American variants might have enhanced oncogenic activity compared to European isolate due to an increased transcriptional activity. Several studies have shown that infections with multiple types of HPV can occur. The majority of multiple infections contain two HPV types, but samples with two, three, four or five types were also seen. The presence of multiple HPV types tended to increase with the severity of cervical disease. Multiple HPV types, usually with at least one type classified as

high-risk, where found in 12% of patients with normal cytology and in 35% of patients with mild moderate dysplasia (Flores *et al.,* 1999).

2.4.3 Parity

High parity has consistently been found to be associated with both cervical cancers (CC) in most case control studies as shown in Figure 4. Furthermore, most of the major studies restricting the analysis to HPV positive women report an increased risk for High Grade Intraepithelial Lesion / Cervical Cancer with increasing number of pregnancies. A Costa Rican study shows that women with seven or more full-term pregnancies had a four-fold increase in the risk of developing squamous-cell CC as compared with nulliparous women (Munoz *et al.,* 2002). Risk of CC significantly increased with increasing number of live births in the large Costa Rica study (Hildesheim *et al.,* 2001). A similar trend was also found among HPV positive women in the Portland study (Schiffman *et al.*, 1993).

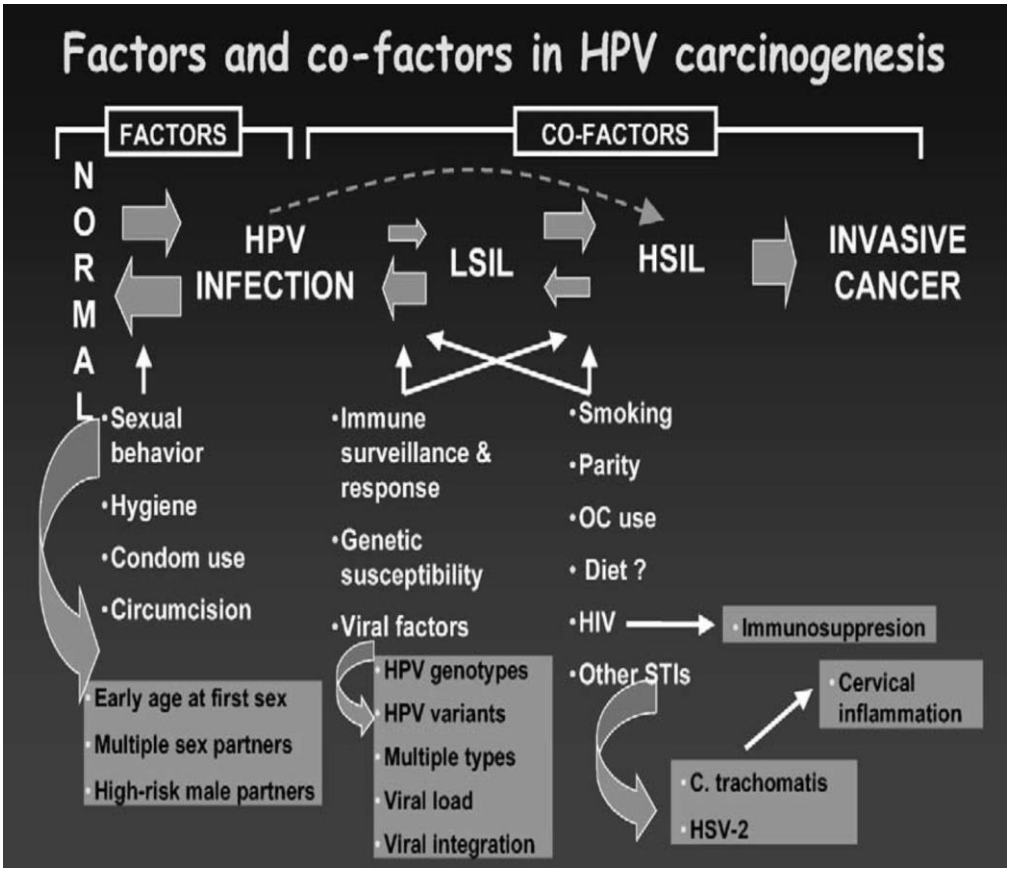


Figure 2.4: Factors and co-factors in cervical carcinogenesis.

Source: Castellsague & Munoz(2003)

Hormonal, traumatic and immunological hypotheses have been put forward as biologically plausible mechanisms to explain the association between parity and cervical neoplasia, but because of the consistency of effects with oral contraceptive (OC) use, hormonal influences are likely to play a role in HPV carcinogenesis. Furthermore, high parity may also increase the risk of CC because it maintains the transformation zone on the exocervix for many years (Autier *et al.*, 1996), facilitating the direct exposure to HPV and possibly other co-factors. Hormonal changes induced by pregnancy may also modulate the immune response to HPV and influence risk of persistence or progression (Munoz *et al.*, 2002).

2.4.4 Oral contraceptives

Use of oral contraceptives (OC) has also been found to be associated with CC in many but not all epidemiological studies. Data from the International Agency for Research on Cancer study among HPV-positive women showed that use of Oral Contraceptives for 5 or more years is a cofactor that may increase up to four-fold the risk of CC among women who are carriers of HPV-DNA (Moreno *et al.*, 2002). Not much data is available concerning the mechanisms by which hormonal influences may modulate the risk of progression to advanced cervical disease among HPV infected women. Hormonal-related mechanisms may influence the progression from pre-malignant to malignant cervical lesions by promoting integration of HPV-DNA into the host genome, which results in deregulation of E6 and E7 expression (IARC, 1995). Experimental studies have shown that estradiol may stimulate the transcription of HPV16 E6 and E7 in cell lines that contain integrated HPV16. Since the E6 and E7 open reading frames have been associated with the oncogenic potential of HPV-16, the effect of oestrogen on the transcription of these viral genes may be of biological relevance in the malignant transformation of HPV-16 infected cervical cells. Data from experimental studies demonstrate a synergistic mechanism between chronic oestrogen exposure and HPV16 oncogenes that modulates squamous carcinogenesis in the female reproductive tract of

transgenic HPV16 expressing mice. Alternatively, Oral Contraceptives might facilitate HPV reactivation or persistence (IARC, 1999; Moreno et al., 2002).

2.4.5 Smoking

Smoking has been related to CC since the late 1970s, based upon the correlations seen between CC incidence and the incidence of other tobacco related cancers. A recent review of the evidence conducted by a Surgeon in the USA retained the hypothesis that a causal association between cigarette smoking and CC was plausible (Public Health Service, 2001).

The fact that nicotine and tobacco-specific carcinogens have been detected in the cervical mucus of smokers (Prokopczyk *et al.*, 1997) strengthens the hypothesis of a synergistic action between cigarette smoking and HPV for the development of CC. Some authors put forward that exposure to tobacco may affect the ability of the host to mount an effective local immune response against viral infections, as it has been shown that smoking may reduce the number of Langerhans cells and other markers of immune function (Poppe *et al.*, 1995).

2.4.6 Sexually transmitted infections

HPV infection with other sexually transmitted agents such as *C. trachomatis* and Herpes Simplex Virus - 2, has inconsistently been associated with CC as shown in Figure 4. In the IARC studies, HSV-2 seropositivity was significantly higher in women with invasive squamous cell carcinoma (44.4%) and adenosquamous carcinoma (43.8%) than in control women (25.6%). Authors postulated that HSV-2 infection may act in conjunction with HPV infection to increase the risk of invasive CC and that this effect is likely to be mediated by the induction of inflammatory responses. Concerning *C. trachomatis,* a large nested case control study of CC, in which HPV exposure was assessed serologically, reported that presence of serum IgG antibodies to *C. trachomatis* serotype G was associated with a 6.6-fold increase in the risk of developing CC as compared with sero-negative women (Anttila *et al.*, 2001). HIV-positive women have consistently been shown to be at increased risk of cervical cancer when

compared with their HIV-negative counterparts and the association appears to be stronger for women with low CD4 T-lymphocyte count. Women infected with both HIV and HPV are at a much higher risk of cervical cancer than women infected with either of the two viruses separately (La Ruche *et al.*, 1998).

2.4.7 Diet and nutritional factors

A number of studies consistently show that higher intakes of fruits and vegetables are associated with reduced risk of cervical cancer. Evidence for dietary vitamin A and blood carotenoids is weakly consistent, whereas the evidence for dietary vitamin C and E is moderately consistent for a reduced risk of developing CC with higher intakes. Epidemiological studies have also been inconsistent regarding a role for folates in the aetiology of cervical neoplasia. Thus, there is moderately consistent evidence showing that higher intakes and blood levels of folates are associated with reduced risk of CC. A recent study found that the dietary intakes of folate, pyridoxine, and cobalamin were inversely related to the risk of developing cervical cancer after adjustment for HPV-DNA and other confounders (Goodman *et al.*, 2001). Although these conclusions suggest that diet may play a role in HPV carcinogenesis, further research using prospective designs and sensitive HPV markers is needed to draw firm conclusions on the relationship between diet, HPV infection and CC risk.

2.4.8 Other co-factors

The role of other potential co-factors is being actively investigated. The IARC studies have recently shown that male circumcision is associated with a reduced risk of genital HPV infection in men and with a reduced risk of CC in women with high-risk sexual partners (Castellsague *et al.*, 2002). Similarly, most studies have shown that the risk of CC is related to age at first sexual intercourse, generally used as a surrogate measure of age at first HPV

exposure. However, definite evidence that CC progression is linked to age at first HPV exposure has not been provided. It has been proposed that the developing cervix or the healing cervix (as a consequence of deliveries, cervical trauma, or any other STD infection) are high risk situations for an HPV infection to reach the basal layer and establish a persistent infection (Bosch *et al.*, 2002).

A recent study conducted in Thailand found that after controlling for HPV type, the risk of developing CC, as compared with the risk of developing intraepithelial lesions, was not related to any of the co-factors considered, except for two indices of socioeconomic status (Thomas *et al.*, 2001). In fact, socioeconomic status is a potential co-factor currently under evaluation (de Sanjose *et al.*, 1997).

2.5 HPV DNA detection

2.5.1 Type-specific PCR

Type-specific PCR assays are based on the sequence variations present in the E6 and E7 genes of HPV subtypes. Fourteen type-specific PCRs for high-risk HPV types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66, and -68) that target approximately 100 bp in the E7 ORF have been developed (Walboomers *et al.*, 1999). Internal control primers are included to detect inhibitory substances. The analytical sensitivity of these assays is between 10 and 200 HPV copies per sample, depending on the HPV type. Type-specific PCRs are currently used primarily in research applications since throughput is limited by the need to use multiple PCR amplifications for each sample.

2.5.2 General primer PCR

The majority of studies using PCR to date have used consensus primers to amplify a broad spectrum of HPV types in a single PCR amplification. These primers target conserved

regions of the HPV genome such as the L1 capsid gene. The MY09 plus MY11 primers target a 450-bp fragment within the HPV L1 ORF (Bosch *et al.*,1995). The GP5+ plus GP6+ primers target a fragment within the region targeted by MY09 and MY11 with an analytical sensitivity of 0.5 to 10 fg (10-200 copies) (Zerbini *et al.*,2001). The MY09 plus MY11 primers failed to detect HPV DNA in 7% of cervical cancers in one study (Volpers& Streek,1991). This may have been due to absence of HPV DNA in the carcinoma cells or a false-negative PCR result due to integration of HPV DNA in the cervical carcinoma which may have disrupted PCR primer target sequences or resulted in loss of the L1 ORF.

Various methods have been used to identify HPV genotypes after amplification with general and consensus primers. Among them are sequence analysis, restriction fragment length polymorphism, and hybridization with type-specific probes using dot blot or micro titer plate formats (Quint *et al.*, 2001).

A PCR-based detection system has recently been developed which uses a general primer set, designated SPF_{10} that that amplifies a 65-bp segment of the L1 region of the HPV genome. Since smaller amplicons are more efficiently amplified, this assay is thought to be especially suited for formalin-fixed, paraffin-embedded tissue samples which often yield poorly amplifiable DNA. Amplicons are detected in an enzyme-linked immunosorbent assay using a mixture of HPV-specific probes that recognize a broad range of genotypes. The specific genotype of positive samples is then determined using a line blot assay, in which oligonucleotide probes are immobilized in parallel lines on nitrocellulose strips. Amplicons are hybridized to the probes on the strip and detected in a colorimetric reaction, which results in a purple precipitate at the positive probe lines (Kleter *et al.*, 1999).

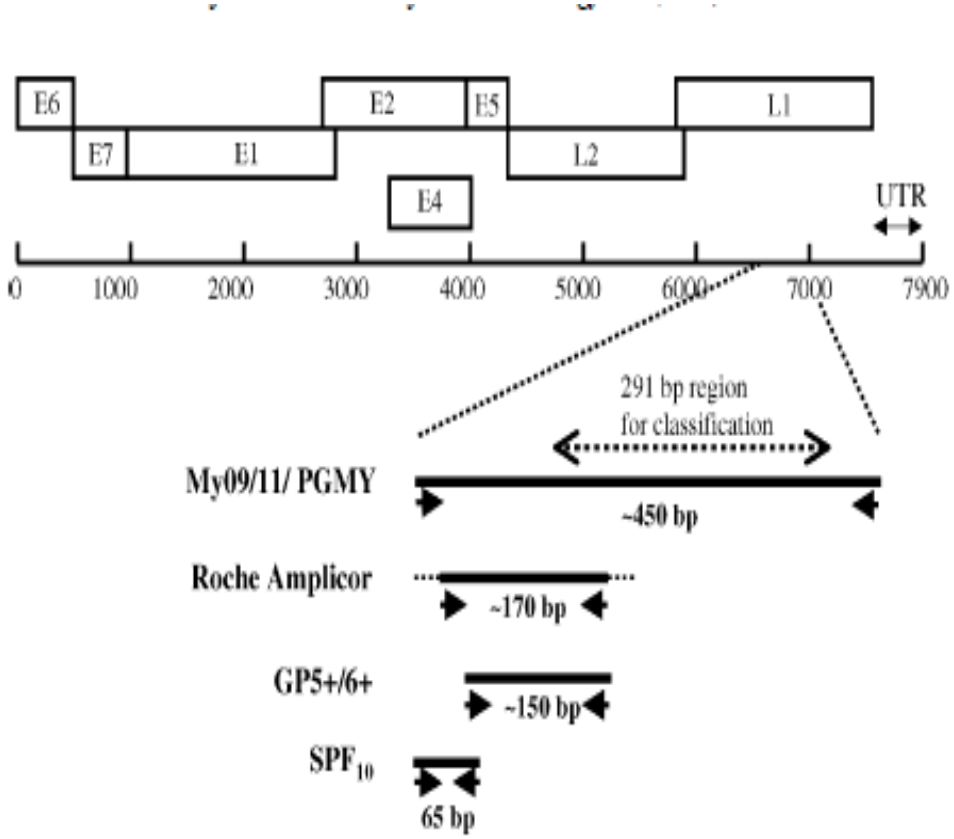


Figure 2.5: Outline of the HPV-DNA genome, presented in a linear form.

The position of the early (E), late (L) genes and the untranslated region (UTR) is indicated, as well as the positions of the four most widely used primer sets CPI/II, MY09/11, GP5+/6+ , SPF10 and Roche Amplicor HPV assay with their respective amplimer sizes (the precise location of the primers, used in the Roche assay is unknown). The 291 bp fragment used for formal classification of HPV genotypes (de Villiers *et al.*, 2004) is shown in the L1 region (Fig 2.5).

The first incorporates one forward and one reverse primer aimed at a conserved region, but fully complements only one or a few HPV genotypes. To compensate for the mismatches with other HPV genotypes, the PCR is performed at a low annealing temperature. The GP5+/6+ PCR system is an example of this approach. The second class of general PCR primers comprises forward and reverse primers, which contains one or more degeneracies to compensate for the intertypic sequence variation at the priming sites. These primers do not have to be used at a lower annealing temperature. The MY09/11 is an example of a degenerated PCR primer set (Hildesheim *et al.*, 1994). In fact, this primer set comprises a complex mixture of many different oligonucleotides. The disadvantage of this design is that synthesis of oligonucleotides containing degeneracies is not highly reproducible and results in high batch-to-batch variation. Therefore, each novel batch of primers should be carefully evaluated to check the efficacy of amplification for each HPV genotype (Molijn *et al.*, 2005).

The third option is to combine a number of distinct forward and reverse primers, aimed at the same position of the viral genome. These primers do not contain random degeneracies, but may contain inosine, which matches with any nucleotide. Using a defined mixture of non-degenerate primers has the advantage that the oligonucleotides can be synthesized with high reproducibility, and PCR is performed at optimal annealing temperatures. Examples of such primer sets are the PGMY primers (Gravitt *et al.*, 2000) and the SPF_{10} primers. Besides the choice of primers, the size of the PCR product is also important.

In general, the efficiency of a PCR reaction decreases with increasing amplimer size. Subjecting clinical samples to treatments, such as formalin-fixation and paraffin-embedding, degrades DNA. Consequently, the efficiency of PCR primers generating a small product is considerably higher than primer sets yielding larger amplimers (Park *et al.*, 2004).

2.5.3 Liquid hybridization

The Hybrid Capture (Digene, Beltsville, Md.) assay is the only kit currently approved by the FDA for the detection of HPV DNA in cervical samples. The Hybrid Capture assay has been used in many studies, and the second-generation Hybrid Capture II version of the assay is now widely used in clinical diagnostic laboratories. It is an antibody capture/solution hybridization/signal amplification assay that uses chemiluminescence detection to qualitatively detect the presence of HPV. In this assay, the DNA in the patient samples is first denatured and mixed with an RNA probe pool in a buffered solution in a tube. Two RNA probe pools are used. The assay can be performed using both probe pools together or separately. The probe A pool recognizes low-risk HPV-6, -11, -42, -43, and -44, and the probe B pool recognizes high-risk HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, and -68. The assay does not distinguish among the HPV types within these groups. Further typing is not clinically significant and is used largely in epidemiology and research studies. The hybridization reaction between specimen target DNA and the RNA probe produces DNA-RNA complexes. The DNA-RNA complexes are immobilized onto wells of a micro titer plate which has been coated with antibody directed against DNA-RNA hybrids. The immobilized hybrids are recognized by a second anti-DNA-RNA antibody conjugated to alkaline phosphatase. Several alkaline phosphatase molecules are conjugated to each antibody, and multiple conjugated antibodies bind to each captured hybrid, resulting in substantial amplification of the signal. Light is emitted as a chemiluminescent substrate is cleaved by the bound alkaline phosphatase. The light is measured as relative light units on a luminometer. The relative light units obtained for each sample are compared to the cut-off value. The analytical sensitivity of this assay ranges from 6.6 to 17.6 pg/ml depending on the HPV type (Digene Corp., Digene HPV test Hybrid Capture II product insert, 1997). The microwell plate format allows the assay to be automated.

Several specimen types can be used in the Hybrid Capture assay. Cervical swabs and cervical biopsy specimens are transported in Digene specimen transport medium. Cervical samples collected in Cytyc ThinPrep PreservCyt solution for making ThinPrep Pap smears can also be used. The Hybrid Capture assay is not intended as a screening assay for the general population. Indications for performing the Hybrid Capture assay include:

(i) To aid in the diagnosis of sexually transmitted HPV infections and to distinguish between infection with low-risk and high-risk HPV types.

(ii) To screen patients with ASCUS Pap smear results so as to determine the need for referral for colposcopy.

(iii) To supplement the Pap smear in women with LSIL or HSIL results to assist in assessing the risk for development of cervical cancer.

The HPV test should not be used independently of Pap smears. A positive HPV result alone does not confirm the presence of premalignant or malignant disease and potentially yields more false-positive results if used on its own (Schiffman *et al.,* 2000).As with any assay, there are some limitations to the Hybrid Capture assay. Cross-reactivity may be observed in rare instances and may lead to false-positive results with the high-risk probe pool. Cross-reactivity may occur in the presence of HPV-13 since both probes cross-react with HPV-13. This finding, however, is not clinically relevant for cervical specimens since HPV-13 is associated with lip lesions in certain ethnic groups and is rarely, if ever, detected in the anogenital tract. A small amount of cross-hybridization can occur between HPV-6 and HPV-42 (low-risk) and the High- Risk probe pool. Specimens with high levels of HPV-6 or HPV-42 DNA (4 ng/ml or higher) may be positive when tested with both probes. Cross-reactivity between both HPV probes and high levels of bacterial plasmid pBR322 which can be found in cervical samples is possible. The false-negative rate is estimated to be 1.1 to 7.5%. False-

negative results can occur due to low levels of infection, sampling error, or the presence of interfering substances such as antifungal cream, contraceptive jelly, or douche.

2.5.4 HPV mRNA Detection

Rather than relying on the detection of different HPV serotypes, the In-Cell (Invirion, Frankfurt, Mich.) viral load test for HPV detects mRNA of the E6 and E7 transforming genes. In this way, the assay actually determines if the HPV genes that causes malignant changes are present and active. The assay can be automated on any analytic instrument that detects fluorescence. Flow cytometry instruments are readily adaptable for this assay by using liquid-based cytology specimens. The assay can also be done directly on Pap smear slides and visualized using a fluorescence microscope. The manufacturer reports that the sensitivity of this assay is 100% and the specificity is 70% compared to Pap smear. Apparent false-positive results account for the reduced specificity; however, these false-positives may in fact not be false but may be due to early up-regulation of the E6 and E7 genes (Burd, 2003).

2.6 Types of PCR method

2.6.1 Real-time PCR

Real-time PCR can also be used to detect HPV-DNA. Type-specific PCR primers can be combined with fluorescent probes for real-time detection, although multiplexing several type specific primers within one reaction can be technically difficult. Broad-spectrum PCR primers have also been used in real-time PCR (Cubie *et al.,* 2001), but are less amenable to quantitation than a type specific primer system. Due to the sequence heterogeneity of different HPV genotypes, genotyping of PCR products from broad-spectrum PCR requires a mixture of probes and since these will all have different hybridization characteristics, standardization is difficult (Hart *et al.,* 2001).

2.6.2 Reverse Transcriptase PCR

It is also possible to look for specific viral RNA by incorporating a reverse transcriptase (RT) step before PCR amplification. Although the vast majority of HPV detection strategies used for epidemiological studies and clinical management have, thus far, been DNA based, detection of expression of HPV oncogenes may have significant clinical value. For example, Lamarcq *et al.* (2002) developed a real-time RT-PCR for HPV 16 and 18 E7 transcripts and suggested that it may be more specific for the detection of symptomatic infections. Wang-Johanning *et al.* (2002) also described an HPV16 E6/E7 quantitative real-time RT-PCR and found that expression increased co-ordinately with severity of the lesion. There is currently one commercially available RNA based HPV assay, the PreTect HPV Proofer (Norchip AS Klokkarstua, Norway).

This assay incorporates NASBA amplification of E6/E7 mRNA transcripts prior to type specific detection via molecular beacons for HPVs 16, 18, 31, 33 and 45. Initial data, on the prognostic value and specificity for underlying disease, is promising (Lie *et al.*, 2004), but the clinical value of this method compared with DNA based assays remains to be determined in large-scale prospective studies (Molijn *et al.*, 2005).

The physical state of the HPV genome has also been explored as a potential diagnostic marker. Integrated virus is associated with a neoplastic phenotype/high grade disease, where loss of the regulatory E2 protein on integration results in up-regulation of oncogenes E6 and E7. Detection of integrated HPV can be performed by identification of viral cellular fusion transcripts such as the APOT technique (Klaes *et al.*, 1999) and by ligation mediated PCR (Luft *et al.*, 2001) with detection of integrate-derived HPV transcripts showing a high specificity for high-grade disease and cancer. However, as application is currently restricted

to identification of types 16 and 18, they are at present more appropriate for epidemiological studies (Molijn *et al.,* 2005).

2.6.3 PCR and restriction fragment length polymorphism (PCR-RFLP)

After amplification, the sequence composition of a PCR product can be investigated by restriction enzymes. Digestion of PCR products with restriction endonucleases generates a number of fragments, which can be resolved by gel electrophoresis, yielding a particular banding pattern. This method is straightforward but labour-intensive. More importantly, the method depends on availability of restriction enzymes capable of detecting specific mutations. Consequently, detection of multiple HPV genotypes, present in different quantities in a clinical sample by PCR-RFLP is usually complex and the sensitivity to detect minority genotypes is limited (Grce *et al.,* 2000).

2.7 Direct sequence analysis of PCR products

Rapid sequencing methods of PCR products are also now available for high throughput, thus permitting application in routine clinical analysis. However, sequence determination is not suitable when a clinical sample contains multiple HPV genotypes. Sequences, which represent a minority species in the total PCR product, may remain undetected.

In turn this may underestimate the prevalence of infections with multiple HPV genotypes, with important consequences for vaccination or follow-up studies. This was confirmed in a recent study from our group comparing sequence analysis of SPF_{10} PCR products with reverse hybridization in 166 HPV-positive cervical scrapes. Compatible HPV genotypes were found in all samples. Direct sequence analysis detected multiple types in only 2% of the samples, while reverse hybridization found multiple types in 25%. The presence of multiple HPV genotypes is a common phenomenon in many patient groups. Up to 35% of HPV-positive samples from patients with advanced cytological disorders and more than 50% of

HIV-infected patients contain multiple HPV genotypes, whereas multiple genotypes are less prevalent in carcinoma patients. The genotype can be deduced from an HPV sequence by two methods (Kleter *et al.*, 1999).

First, the sequence can be used to interrogate a sequence database using a homology search. Extensive databases are available on the Internet and can be freely accessed at http://www.ncbi.nlm.nih.gov. BLAST software permits fast homology searches of a sequence within a continuously updated sequence database (Altschul *et al.*, 1990).

Secondly, phylogenetic analyses can be performed. The novel sequence can be used in a multi-sequence alignment with a set of known HPV sequences, representative of different HPV genotypes. Based on the sequence alignment, a phylogenetic tree can be constructed, providing a graphical representation of the evolutionary relationships between the detected sequence and reference sequences, and a genotype can be deduced. It should be noted that formal classification of genotypes is entirely based on sequence analysis of the viral genome, whereas genotyping of clinical samples is performed by analysis of only a limited, but representative part of the genome (Molijn *et al.*, 2005).

2.8 Prevalence of HPV

The prevalence rate of cervical cancer among women in Nigeria is 26.3%, west Africa is 21.5% and world prevalence rate 11.4% (Thomas 2004, de Sanjos 2010), The incidence rate of cervical cancer in Nigeria is 25/100,000 while the report prevalence rates for HPV among women is 26.3% by Thomas *et al.*, (2004) and 24.0% by Okolo *et al.*, (2010) respectively. A prevalence rate of 37% was also recorded by Akarolo- Anthony*et al.*, (2014) in a study carried out within two hospitals in Abuja. Fadahunsi *et al.*, (2013) also recorded a high- risk HPV prevalence rate of 21.6% among women in Ile-Ife Ibadan. Kolawole *et al.*, (2015) recorded a prevalence rate of 4.93% among women who had abnormal cytology in Lokoja.

High risk HPV types 16, 31, 35, 56 were found with infections involving more than one HPV type and high prevalence of HPV in all age groups. There is a high burden of cervical cancer with mean age of patients of 52.4 years within the Federal Capital Territory, (FCT). Age in affected women was between 12-19 years, with an average age of 15 years and 86% of patients presenting late (Nnodu *et al.*, 2010).

Estimates of HPV prevalence is about 20 million, with at least an annual 5.5 million new HPV infections. The global prevalence of HPV infection is 11.7%, Sub – Saharan Africa is 24%, Eastern Europe is 21.4%, Latin America is 16.1% and South-eastern Asia 14.0% (IHPRC, 2015).

The HPV annual incidence compares to their estimates of 3 million Chlamydia infections annually, one million Herpes infections annually, 650,000 Gonorrhea infections annually, and 20,000 HIV infections annually. The estimate of HPV prevalence of 20 million current infections compares to 45 million Herpes current infections, two million Chlamydia current infections, 750,000 Hepatitis B current infections, and 560,000 HIV current infections. The estimates of this study suggest that HPV is the fastest growing STD in the world (CDC, 2008).

The overall prevalence of HPV in the studied Population in Mexico in 2009 was 25.4%. As expected, the highest HPV prevalence was found in women with Cervical Cancer (87.5%), followed by women with HGSIL (29.4%), LGSIL (24.0%), and normal cytology (14.7%), (Marquez *et al.*, 2009). CDC conducted a study in which they investigated HPV prevalence among women from the University of California, Berkeley. A total of 467 women were enrolled in the study, with a mean age of 22.9 years old. Of this sample, 213 (46%) of the women tested positive for HPV. Women who were 22 to 25 years old were the most likely to have HPV (22 to 23: 54.8%; 24 to 25: 51.7%). Black women were most likely to have HPV, while Asian women were least likely to have HPV. These findings have been shown by other

researchers such as Winer *et al.* (2006) who found HPV infection prevalence in university females to be 37.2%. Winer *et al.* (2003) investigated HPV prevalence in the Washington state area of young women ages 18 to 20. These researchers enrolled participants for four months follow ups for approximately three years from 1990 to 1997. Participants were tested for HPV and behavioural and medical information was updated at each visit (every four month). There was a total of 553 women who came for a first visit, 109 (19.7%) of whom tested positive for HPV. The cumulative 24 months' follow-up resulted in 38.8% of the women been positive for HPV. These women had a mean age of 19.2 (SD = 0.5).

Other researchers have found a much higher prevalence of HPV infection among the late teens early twenties age group. Moscicki*et al.* (2001) found that out of 105 young women in the San Francisco area (mean age = 20.0, SD = 1.9), there were 54 cases of HPV infection. Of these infections, 46% were high risk HPV types only, 9% were with low-risk types only, and 13% were mixed. Winer *et al.* (2003) had a much lower percentage of high-risk HPV types (20.6% of the total HPV infections). HPV infection tended to go away after about a year (70% were no longer infected after 12 months), and the median HPV infection time was eight months.

HPV infection has also been shown to be highly prevalent in the HIV community. Sun *et al.* (1997) used examinations of 787 HIV-seropositive women and 721 HIV-seronegative women in New Orleans to investigate the prevalence of HPV infection in this sub-population. Results showed that at initial examination, 56% of the HIV-seropositive women and 31% of the HIV-seronegative women tested positive for HPV. The cumulative HPV prevalence was 83% in the HIV-seropositive women and 62% in the HIV-seronegative women (testing went from 1991 to 1993). The HIV-seropositive women were significantly more likely, to test positive for HPV at subsequent visits and have persistent HPV infections of the same strand. These

finding were similar to Winer*et al.*'s (2006) results in that HPV infection consistently went down over time, indicating that the average HPV infection may not last long.

HPV infection has been suggested to be the fastest rising STD in the United States of America. HPV is very high among the college student age group of around 18 to 22 years. Studies have consistently shown an infection prevalence of around 20% to 50% of females in this age group to test positive for HPV (Moscicki *et al.,* 2001; Winer *et al.,* 2003; Winer *et al.,* 2006;).

2.9 Clinical manifestations of HPV in women

Once HPV has infected any region of the epithelium of the interior genital tract, in approximately 80% of the cases the organism will totally be eliminated, while in the rest of the cases the virus may remain latent, even for decades, in which case, it is only possible to diagnose through molecular biological methods, which detect the presence of viral DNA, or rather enter into a stage of active expression with morphological manifestations depending on the induced histology in the epithelium, which present as clinical or sub-clinical manifestations (Guerra-Tapia *et al.,* 2009). The clinical forms are generally benign, while the subclinical may be expressed as benign lesions or precursory lesions with the potential to develop malignant lesions. Although the clinical lesions are evident with direct ocular examination, broadened examination through colposcope allows more evaluation of the extension of the disease and better evidence of its clinical aspects, on which a correct therapeutic plan will depend. On the other hand, sub-clinical lesions are only evident on colposcopic examination after applying 5% acetic acid. These lesions may affect any area of the interior genital tract and the simultaneous existence of mixed forms of clinical and sub-clinical manifestation is frequent (Guerra-Tapia *et al.,* 2009).

Clinical lesions are seen as genital warts, which macroscopically appear to be made of a series of papilla of fleshy appearance, which sprout from a common root, more frequently,

extensive and keratinised on the cutaneous surface than on the mucous membranes, with a characteristic cockscomb or cauliflower appearance and with a highly variable size and extension. A very infrequent form is the giant condyloma, in general benign, although it is sometimes seen with malignant histology of the verruciform carcinoma, known as the Buschke-Lowenstein tumour. Rounded, slightly raised, papular forms may also be seen in the vulva, which whiten with acetic acid and are sometimes pigmented (bowenoid papulosis), in which case a possible serious preneoplastic lesion (*in situ* carcinoma), or macular lesions, reddish or grey-white, which generally affect the mucous membranes should be suspected. Condylomas are nearly always benign, although 5% may contain oncogenic virus, while the papules and macules may have HPV's without important histological repercussions to serious precursory lesions, and should, thus, always have a biopsy performed on them. The sub-clinical lesions are found on the majority of the mucous membranes and are seen as white coloured stains, which appear after the application of acetic acid and should be interpreted by a specialist. In the cervix, infection by HPV may affect the squamous epithelium or the joining area between the glandular and squamous epithelium in the same way. Outside these, the epithelia express either a sub-clinical HPV infection or abnormal epithelium with defects in maturity and benign character, while within these, apart from the changes referred to, they may indicate HPV-associated preneoplastic lesions with various grades of severity, and as such should undergo biopsy for histological confirmation and be evaluated by a specialist, who, depending on the morphological form, will decide on the most convenient places for taking the biopsy (Guerra-Tapia *et al.,* 2009).

2.10 Diagnosis

Pap test: during this test, the health care provider removes a sample of cells from the cervix. The cells are then stained and examined under a microscope to look for any changes in the

cells, even in the absence of genital warts. It is advisable for women to go for Pap smear screening every 3 years and yearly for women with certain risk factor (NCI, 2012).

Colposcopy: for this test, a health care provider uses an instrument called a colposcope that shines a light and magnifies the view of the cervix. A vinegar solution is placed on the cervix. The solution turns abnormal cells that are infected with HPV white, so they can be more easily seen. This method of diagnosis is not specific as a result; further analysis using HPV DNA is required to identify the various subtypes (NCI, 2012)

HPV DNA test: this test looks directly for the genetic material (DNA) of the HPV within a sample of cells. The test can detect the type of HPV connected to cervical cancer. The sample used for this test is generally removed at the same as a Pap test. This method of diagnosis is molecular and very specific as it identifies the different subtypes of HPV (NCI, 2012).

HPV Serology: this involves the use of Enzyme Linked Immunosorbent Assay (ELISA) technique to determine the level of HPV antibodies in the Serum/Plasma of an individual. This test is very sensitive as it detects the smallest amount of antibodies present (CDC, 2008).

2.11 Treatment

Most HPV-induced cervical cell changes are transient, and 90% regress spontaneously within 12 to 36 months as the immune system eliminates the virus. The primary immune response to HPV infection is a cell-mediated response induced at local lymph nodes. 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 (Bontkes *et al.,* 1999)**.**Systemic levels of HPV-specific IgA were correlated with virus clearance. Systemic levels of HPV-specific IgG were detected more frequently in patients with persistent HPV infection. The tendency toward regression of HPV infection correlates inversely with the severity of cervical disease. Only a

small proportion of mild and moderate cervical diseases develop into invasive cancer, but the risk of progression from severe cervical cellular abnormality to invasive carcinoma is at least 12% **(**Ostor, 1993). Factors such as genetic predisposition, frequency of re-infection, intra-typic genetic variation within HPV type, co-infection with more than one HPV type, hormone levels, and immune response may influence the ability to clear an HPV infection.

A number of factors such as size, stage, and histologic features of the tumour, lymph node involvement, and risk factors for complications from surgery or radiation, and patient preference determine the course of treatment. In general, non-invasive intraepithelial lesions identified only microscopically are treated with superficial ablative procedures such as cryotherapy or laser therapy. These are outpatient office procedures, and fertility is maintained. With cryotherapy, abnormal tissue and the surrounding 5 mm is frozen with a super cooled probe. A single freeze is usually not adequate to induce necrosis, so the area is allowed to thaw and is frozen again. Ablation of tissue with a carbon dioxide laser beam is as effective as cryotherapy, and the tissue heals faster with less distortion, but the procedure is more expensive. Loop electrosurgical excision procedures are now considered to be the preferred treatment for non-invasive squamous lesions. In these procedures, an electrically charged wire is used to excise the transformation zone and distal endocervical canal. It is less expensive than laser therapy and preserves the excised tissue for histologic examination of margin status.

Following treatment of non-invasive intraepithelial neoplasia lesions by any technique, there is always a potential risk of leaving dysplastic cells behind. Recurrence rates as high as 31% with a mean time to recurrence of 9 months have been reported following loop diathermy procedures in immunologically normal patients. Patients with positive margins had a higher recurrence rate (47%) than did those with clear margins (26%). Human immunodeficiency

virus-infected women have a significantly higher recurrence rate (87%) than do uninfected women (18%), indicating the importance of an effective immune system in resolution of HPV-associated disease (Calore *et al.*, 2001**).** Progression to invasive disease is rare (< 2% in most series). However, these data emphasize the importance of follow-up surveillance in treated patients. Preliminary evidence suggests that detection of HPV DNA using molecular techniques may be able to help detect residual lesions following treatment **(**Nobbenhuis *et al.*, 2001**)**. Detection of high-risk HPV DNA at 6 months after treatment was more sensitive than abnormal cytology findings in patients with moderate or severe cervical disease prior to treatment. The negative predictive value of absence of high-risk HPV DNA and normal cytologic test results in these patients was 99%. The utility of HPV DNA testing for residual disease following treatment of lower grades of dysplasia remains to be evaluated. Micro invasive cancers less than 3 mm in size are managed conservatively by excisional cone biopsy.

Early invasive cancers are managed with radical hysterectomy or external-beam high-energy (to 18 MV) radiotherapy and implants loaded with ^{192}Ir. The goal of this therapy is to destroy malignant cells in the cervix, paracervical tissues, and regional lymph nodes. Selected patients also benefit from concurrent chemotherapy.

Locally advanced cancers are managed with radiotherapy to the primary tumour and potential sites of regional spread. In addition to surgical and cyto-destructive procedures, several antiviral and immunomodulatory agents have been evaluated as treatment for HPV-associated cervical lesions. Cidofovir is an acyclic nucleoside phosphonate derivative which has broad-spectrum activity against DNA viruses and is in use clinically for the treatment of CMV infections. Exposure of human carcinoma cell lines containing HPV-16 or HPV-18 and human cervical keratinocytes immortalized by HPV-33 to cidofovir resulted in inhibition of

cell proliferation. The in vitro antiproliferative activity was shown to be selective for the rapidly proliferating HPV-infected cells when normal primary human cervical keratinocytes were treated similarly. A 1% cidofovir gel was used topically without side effects every other day for 1 month to treat 15 women with severe CIN (Snoeck *et al.*, 2000). Complete or partial response was seen in 80% of patients as assessed by histology and detection of HPV DNA by PCR.

Podophyllin, a cytotoxic agent that arrests mitosis in metaphase (also used to treat genital warts), in combination with vidarabine, a DNA polymerase inhibitor, suppressed HPV gene expression and cell growth in cervical cancer cell lines **(**Okamoto *et al.*, 1999). The expression of HPV-16 E6 and E7 gene products in normal cervical keratinocytes in vitro in the presence of either podophyllin or vidarabine sensitized these cells to apoptosis. Combined topical therapy with podophyllin and vidarabine ointments in 28 patients with mild to moderate CIN resulted in regression of lesions and successful eradication of HPV-16 or HPV-18 DNA in 81% of patients.

The IFNs and intravaginal 5-fluorouracil have shown variable response in clinical and in vitro studies. IFN-α is approved for treatment of genital warts. The effects of IFN-α, IFN-β, and IFN-γ in several human carcinoma cell lines containing HPV-16 or HPV-18 have been studied. Response was seen in some cell lines but not others. In HPV-18 HeLa cells, all IFNs suppressed the levels of HPV E6 and E7 gene transcripts. In HPV-18 C-411 cells, IFNs had no effect. In HPV-16 CaSki and HPK1A cells, only IFN-γ was effective. It is likely that, since IFN-responsive elements appear to be down-regulated by at least some oncogenic HPV types, the utility of IFN therapy in cervical disease will be limited (Chang & Laimins, 2000).

2.12 Prevention and Control

Primary approaches to prevent HPV infection include both risk reduction and development of HPV vaccines. Use of latex condoms and a spermicide may decrease the risk of contracting HPV. Condoms, however, are not totally reliable, since HPV may be contracted by contact with other parts of the body, such as the labia, scrotum, or anus that are not protected by a condom.

Vaccines directed against HPV are in phase I and phase II clinical trials but are not currently commercially available. HPV vaccines are usually composed of virus-like particles (VLPs), which are empty virus capsids containing the major HPV capsid antigen and possibly the minor capsid antigen but lacking viral DNA. The vaccines are produced by expressing the L1 or L1 and L2 ORFs in eukaryotic cells. These proteins then self-assemble into VLPs which are highly immunogenic. Because of the high level of antigenic specificity of HPV capsid antigens, there is no cross-protection among subtypes, and protection against each subtype requires vaccination with VLPs of that subtype. Optimal vaccines would contain a cocktail of VLPs of the most common high-risk HPV subtypes. Preliminary reports indicate that animal papillomavirus vaccines have excellent immunogenicity and protection against experimental animal papillomavirus diseases.

A double-blind, randomized, placebo-controlled phase I safety and immunogenicity trial has been conducted using a subunit vaccine composed of VLP formed from the entire L1 major capsid protein of HPV-16 strain 114K **(**Harro *et al.*, 2001).The vaccine was prepared by inserting the L1 capsid gene into a baculovirus vector. The gene was then expressed in transfected Sf9 insect cells. An optimal dose of 50μg of HPV-16 L1 VLP vaccine was administered by injection into the deltoid muscle at 0, 1, and 4 months. The vaccine

generated high titres of type-specific neutralizing antibodies without adjuvant and was well tolerated.

Cancer control describes the totality of activities and interventions that are intended to reduce the burden of cancer in a population either by reducing cancer incidence or mortality or by alleviating the suffering of people with cancer. Prevention, early detection, diagnosis, treatment, psychosocial support, and palliative care are components of cancer control that can reduce the cancer burden. Nigeria's Cancer Control Plan 2008 to 2013 is aimed at providing information and education through outreach services nationwide (Nnodu *et al*, (2010).

Because the HPV virus is so contagious, total prevention of the HPV virus is not easy. Abstinence of all sexual contact, even skin to skin sexual activity without penetration, is the only way to avoid contracting the HPV virus.

Lifelong monogamy (having one long term sexual partner) is another effective way to avoid contracting the HPV virus. Limiting the number of partners is advised because the more sexual partners you have, the higher your risk of contracting HPV.

Regular Pap testing is the only way to detect abnormal cells in your cervix that could lead to cervical cancer later in life. A woman should have a Pap test within three years of becoming sexually active, and then repeat testing every two years (SOGC, 2007).

2.13 Vaccines in place

Vaccines have been developed to prevent you from contracting the HPV virus and could dramatically help reduce the incidence of HPV related complications such as genital and anal warts and cervical cancer. In Canada, there are two vaccines available on the market: one protects against types 16 and 18 and the other against types 6, 11, 16 and 18 (SOGC, 2007). The two prophylactic vaccines prevent cervical cancer but do not cure existing infections.

They are based on the LI virus-like particles that are required to achieve immunity against HPV. The LI protein is capable of self-assembling to form empty virus like particles which activate the human immune system from antibodies. The HPVs targeted by the vaccines are “high risk’’ types 16 and 18 and ‘’low risk’’ type 6 and 11. The two commercial HPV vaccines are Gardasil, manufactured by Merck, and Cervarix, manufactured by GlaxoSmithKline. Both are made using genetically modified (GM) microbes in a laboratory.

Gardasil protects against all four HPV types because it contains virus like particles with mixtures of the four sub-unit protein, and is called a tetravalent vaccine. The vaccine contains an aluminium adjuvant. Protection requires a first inoculation and booster shots at 1 and 6 months after the first. The four LI proteins are manufactured using GM baker’s yeast.

Cervarix protects against the HPV types 16 and 18, and is a bivalent vaccine containing an aluminium adjuvant along with a compound called 3-O-deacylated-4’- monophosphoryl lipid A. Vaccination is repeated at 1and 6 months after the first injection. The vaccine is manufactured using GM baculovirus produced in cultured insect cells (Schiller *et al*, 2008).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study area

Abuja is the Federal Capital of Nigeria and it is located in the central part of the country. The Federal Capital Territory (FCT) was carved out in 1976 from parts of Nasarawa, Niger, and Kogi States all within the central part of Nigeria. It has land area of 7,315 square km and human population of 979,876 (an estimate as at 2012). Its geographical coordinates are 9°4′0″N7°29′0″E. The territory is bordered by Kaduna to the North-East, Plateau to the East and South, Kogi to the South-West, and Niger to the West and North-West (Abumere, 1984).

The FCT is divided into six administrative units namely; Abuja Municipal, Abaji, Gwagwalada, Kuje, Bwari and Kwali. Abuja has a tropical wet and dry climate with an annual total rainfall of 43.3 inches (1100 mm) to 63 inches (1600 mm). The indigenous tribes include Gwari, Koro, Ganagana, Gwandara, and Bassa. Farming is the major occupation of the indigenous tribes (Murray, 2007).

Figure 3.1: Map of Nigeria showing the study area FCT

Source: (Abumere, 1984; Elleh, 2001; Murray, 2007).

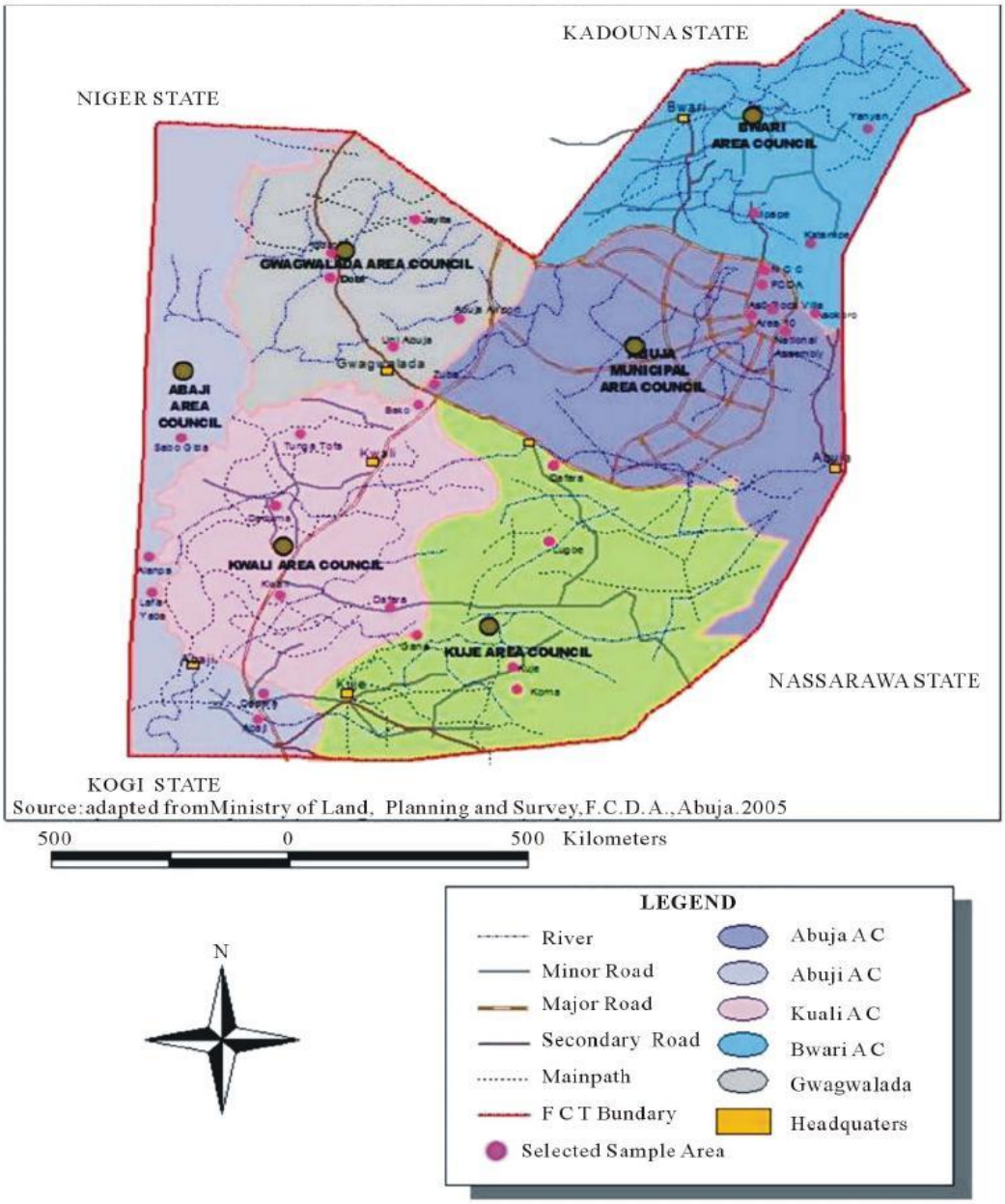


Figure 3.2: Study sites in the Federal Capital Territory

Source: (Ministry of Land, Planning and Survey, F.C.D.A, Abuja, 2005).

3.2 Study Design

The study was a hospital based, where cervical swab samples were randomly collected from women seen at the outpatient department (G.O.P.D) and the Gyneacology Department of General hospitals Abaji, Bwari, Kwali, Kuje, Nyanya and Asokoro District Hospitals within the Federal Capital Territory.The choice of the hospitals was purposeful, because the hospitals serve as referral centres for most primary health care centres in the Federal Capital Territory.

3.3 Sample size determination

The sample size for this study was determined using the method described by Magnani, (1997) in theEquation.

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

Where n = sample size

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

p = prevalence rate

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

To calculate n using the 12.4% prevalence obtained by Okwuraiwe *et al.*, 2015.

See details below:12.4% = 0.124

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

$$n = \frac{1.96^2 \text{ x } 0.124(1\text{-}0.124)}{0.05^2}$$

$$n = \frac{3.8416 \text{ x } 0.124(0.876)}{0.0025}$$

$$n = \frac{0.4172899584}{0.0025}$$

$$n = 166.91598$$

$$n = 167$$

Since larger sample size give minimal errors statistically (Robert *et al.*, 2014), 167 was multiplied by 3 to give 501.

3.4 Study Population

The study involved 501 women attending the Obstetrics and Gynecology Clinic of the selected hospitals (Asokoro district hospital (84), General hospital Abaji (83), Bwari (83), Kwali (83), Kuje (84), and Nyanya (84). Structured questionnaire was administered to the women, after an informed consent (Appendix II) had been obtained from the women.

3.5 Ethical Approval and consent

Ethical approval (Appendix I) for this study was obtained from the Research and Ethics Committee of the Federal Capital Territory Administration, Abuja, Nigeria.

3.6 Inclusion and Exclusion Criteria

Inclusion criteria:

Women who were attendees of the selected hospitals and were within the age group of 15 to 65 years and had given their consent were included in the study.

Exclusion criteria:

Women who were not attendees of the selected hospitals and were below 15 or above 65 years were excluded. Similarly, women who refused to give their consent were also excluded from the study.

3.7 Collection of Demographic data

A structured questionnaire (Appendix III) was administered to individuals who gave their consent. English and other local dialects were used where necessary to aid in acquiring needed socio demographic information of the participants.

3.8 Sample Collection and Preparation

Cervical smears were collected from 501 women attending the selected hospitals. The samples were collected as follows; each of the participants was placed in a dorsal position, with her legs flexed at the hip and knee abducted. The labia were parted with gloved thumb and index fingers. A Cusco's bivalve speculum which is not lubricated was passed and fixed to visualize the cervix, under a bright light source. The detachable end of the cervical brush; Rovers® Cervex-Brush® cell sampling device (Rovers Medical Devices B.V 5347 KV Oss, The Netherlands) was then inserted into the cervix and rotated through 360^{o} movements, either in a clockwise or counter clockwise direction, to scrape the entire squamocolumnar junction of the transformation zone. The brush was then inserted into the vial containing preservative fluid. Liquid-based cytology system (Liqui- PREP by LGM International, Inc, Melbourne, FL, USA) for collection and transport of cervical specimen was used.The specimen was stored at -20^{o}C for further analysis (Mbamara *et al.*, 2011).

3.8.1 Sample Preparation

The stored samples(-20^{o}C) were transported under frozen condition to a DNA laboratory in Kaduna for ELISA screening and molecular analysis (Salvatore *et al.,* 2010).

3.9Detection of HPV using Enzyme Linked Immunosorbent Assay (ELISA)

3.9.1 Principles

ELISA method is developed for the detection of antigen or antibody. It consists of use of corresponding antibody or antigen in question which is firmly fixed on solid phase, such as plastic surface of polyvinyl plate or polystyrene tube. The test sample is then added in the microtitre plate, if there is presence of Antigen or Antibody in the test sample, there will be Antigen-Antibody reactions. Enzyme labelled antibody is added in the reaction mixture, which will combine with either test antigen or test antibody.

The enzyme system consists of an enzyme (horse radish peroxidase, alkaline phosphatase which is labelled or linked, to a specific antibody) and specific substrate: O-Phenyl-diamine-dihydrochloride for peroxidase and P Nitrophenyl Phosphate- for Alkaline Phosphatase. Substrate is added after the antigen-antibody reaction. The enzyme catalyses (usually hydrolyses) the substrate to give a color end point (yellow compound in case of alkaline phosphatase). The intensity of the color is proportional to the amount of antibody or antigen present in the test sample, which can be quantified using ELISA reader.

3.9.2 Procedure

All reagents and samples were brought to room temperature of 28^{o}C for 30mins before commencing the assay. The numbering of the samples was done in sequence to correspond with the microtitre plates. The positive and negative controls were centrifuged at low speed for two seconds.Positive and negative controls and sample wells were set, then 50µl of positive control was added to the positive control and negative control wells. Fifty

microlitresof each sample was added to each sample well.One hundred microlitres of HRP-conjugate reagent was added to positive and negative control wells plus other wells. The plates were covered with an adhesive strip and incubated for 60 minutes at 37 °C. The plates were then washed using the wash solution four times. Fifty microlitres of chromogen solution A and 50µl chromogen solution B was added to each well. It was thoroughly mixed and then incubated for 15 minutes at 37 °C. Fifty microlitres of stop solution was added to each well. The change in color was observed in the wells. Results were read at an Optical Density OD of 450 nm using an ELISA reader within 15 minutes. The cut-off value was given as the average of the negative wells plus 0.15 (as specified by the manufacturer). Any sample whose optical density (OD) was less than the calculated cut-off value was interpreted as having a negative result while samples with higher optical density than the calculated cut-off value was interpreted as been HPV positive result (Manufacturer instructions).

3.10 DNA Extraction Procedures

Bioneer Accuprep Genomic DNA Extraction Kit (K-3032) was used for the extraction of the HPV DNA as follows:Proteinase K was mixed with 1,250µl of deionized water and then 20µl of the solution was pipette into each labeled Eppendorf tube. Then, 200µl of each sample was added to the tubes containing Proteinase K and then 200µl of GB (Binding) buffer was added to the mixture. It was mixed thoroughly. It was then lubricated at 60°C for 10 minutes. Then 400µl of ethanol was added and mixed. The lysate was carefully dispensed into the upper reservoir of the binding column tube (which is fit in a collection tube). It was then closed and centrifuged at 8000 rpm for 1 minute (Bioneer.us.com, 2018).The solution from the collection tube was then discarded and the collection tube placed back under the binding column tube for further analysis. Exactly 500µl of washing buffer one (WA1) was added, the tube was closed and then centrifuged at 8000 rpm for 1 minute. The solution from the collection tubes were discarded so as to reuse the collection tube again. Then 500µl of

washing buffer two (W2) buffer was added, the tubes were closed and centrifuged at 8000 rpm for another 1 minute and the solution from the collection tubes were discarded. This was centrifuged once more at 13000 rpm for 1 minute to completely remove ethanol and droplet clinging to the bottom of the binding column tubes (Bioneer.us.com, 2018).The binding column tubes were transferred to a new Eppendorf tubes for elution. Fifty microlitres of EA buffer was added to the binding column tubes and kept for 1 minute at 28°C after which the mixture was centrifuged at 8000 rpm for 1minutes to elute into the Eppendorf tubes. The eluted DNA samples were then stored at -20°C for amplification.

3.11 DNA amplification using Polymerase Chain Reaction technique

3.11.1 Principles

PCR uses the enzyme DNA polymerase that directs the synthesis of DNA from deoxy nucleotide substrates on a single-stranded DNA template. DNA polymerase adds nucleotides to the 3` end of a custom-designed oligonucleotide when it is annealed to a longer template DNA. Thus, if a synthetic oligonucleotide is annealed to a single-stranded template that contains a region complementary to the oligonucleotide, DNA polymerase can use the oligonucleotide as a primer and elongate its 3` end to generate an extended region of double stranded DNA (Joshi *et al.,* 2011).

3.11.2 Procedure

All the positive ELISA specimens were amplified HPV DNA using nested Polymerase Chain Reaction (nPCR) with PGMY 09/11 and GP5+/GP6+ consensus primers which amplifies a 450 bp and 150 bp fragment of the L1 HPV genomic region respectively (Gravitt *et al.,* 2000). Accu- Power HotStart Premix K-5051 (Bioneer Corporation, USA) was used for the PCR and Thermal cycler (Bio Rad) was used for PCR.

The PCR kit contained PCR premix solution for a 20µl reaction.

Primers used were PGMY09 and PGMY11:

PGMY11-A- GCACAGGGACATAACAATGG

PGMY11-B- GCGCAGGGCCACAATAATGG

PGMY11-C- GCACAGGGACATAATAATGG

PGMY11-D- GCCCAGGGCCACAACAATGG

PGMY11-E- GCTCAGGGTTTAAACAATGG

PGMY09-F- CGTCCCAAAGGAAACTGATC

PGMY09-G- CGACCTAAAGGAAACTGATC

PGMY09-H- CGTCCAAAAGGAAACTGATC

PGMY09-Ia- GCCAAGGGGAAACTGATC

PGMY09-J- CGTCCCAAAGGATACTGATC

PGMY09-K- CGTCCAAGGGGATACTGATC

PGMY09-L- CGACCTAAAGGGAATTGATC

PGMY09-M- CGACCTAGTGGAAATTGATC

PGMY09-N- CGACCAAGGGGATATTGATC

PGMY09-Pa- GCCCAACGGAAACTGATC

PGMY09-Q- CGACCCAAGGGAAACTGGTC

PGMY09-R- CGTCCTAAAGGAAACTGGTC

HMB01b- GCGACCCAATGCAAATTGGT

Gp5+: 5′TTTGTTACTGTGGTAGATACTAC3′

Gp6+: 5′GAAAAATAAACTGTAAATCATATTC3′

The procedure involved:

Sixteen microliters (16µl) of deionized water were added to each tube containing Hot-start PCR Premix while 18µl of deionized water was added to the negative control tube. Then 2µl of the PGMY primer was added to each tube followed by 2µl of DNA template. This was then mixed to ensure that every component settles at the bottom of the tube. This was inserted into the thermal cycler to run for 35 cycles under the following conditions:

Pre – denaturing at 94°C for 5 minutes, denaturing at 94°C for 1 minute, Annealing at 45°C for 1 minute, Extension at 72°C for 1 minute and final Extension at 72°C for 5 minutes.

Upon completion, a second round was initiated using the end product of the first round. The process involved;

Sixteen microlitres of deionized water were added into a fresh hot-start premix, 2µl GP5+/6+ primer and 2µl of the first-round end product. This was mixed and inserted into the thermal cycler to run for 30 cycles using the following condition:

Pre – denaturing at 94°C for 5 minutes, denaturing at 94°C for 30 seconds, Annealing at 40°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 5 minutes. The final product was stored for further analysis. Presence of an amplicon at 150bp position indicates a positive result (Bioneer.us.com, 2018).

3.12 Detection of PCR products using Gel Electrophoresis

3.12.1 Principles

Agarose gel electrophoresis is a routinely used method for separating DNA. Nucleic acid molecules are size separated by the aid of an electric field where negatively charged molecules migrate toward anode (positive) pole. The migration flow is determined solely by the molecular weight where small weight molecules migrate faster than larger ones. In addition to size separation, nucleic acid fractionation using agarose gel electrophoresis can be an initial step for further purification of a band of interest. Extension of the technique includes excising the desired "band" from a stained gel viewed with a UV Tran illuminator (Yılmaz *et al.,* 2012).

3.12.2 Procedure

Exactly 1.5g of Agarose powder (QD LE Agarose, Green Bioresearch, USA) was weighed in a conical flask and dissolved in 100mls of Tris Acetate EDTA (TAE) buffer. The mixture was heated in a microwave for five minutes to dissolve. This was allowed to cool in a water bath, set at 50^{o}C. The gel casting tray was set and combs was placed in the gel tray. A 12µl of ethidium bromide was added to the cooled gel, rocked gently and poured into the gel tray. This mixture was then allowed to set for 45minutes, at room temperature. The comb was then removed and placed in electrophoresis chamber and flooded with Tris-Acetate-EDTA (TAE) buffer. About 10µl The PCR product was mixed with 1µl loading dye (bromophenol blue) and dispensed into various wells on the gel. While 8µl molecular maker (ladder) and the negative control was loaded onto gel and this was electrophoresed at 100 volt for 40 minutes. The gel was removed and excess buffer removed by mopping on a paper towel. This was then exposed to UV light with a gel documentation system. The DNA bands were visualized, using a gel imaging system (Gel Doc 2000, Bio-Rad Laboratories Inc., Hercules, CA, USA).

The result was determined by extrapolating from the amplicon band and the ladder band (Bioneer.us.com, 2018).

3.13 Optimization Reaction

The second round PCR was repeated followed by gel electrophoresis for optimization. The maker and PCR product were dispensed into five PCR tubes instead of one. This was done to have more concentration and then placed in a thermo cycler for second round cycling conditions. The marker and PCR products were placed in the respective wells and gel exposed to electromotive force of 100 volts for 40mins. The gel was exposed to UV light and pictures taken with a gel documentation.

3.14 DNA Clean Up for Sequencing Reaction

The excision of DNA fragments from the Agarose gel was done and placed in 1.5ml micro tubes and weighed. A 3x gel volume buffer GB (gel extraction buffer) was then added. The samples were incubated using heating block (BioBlock Scientific, Thermolyne Corporation, Iowa, USA) for 10mins at 50°C. The samples were vortexed (Vortex mixer- Vision Scientific Co. Ltd, Korea) for 2 mins to properly dissolve. A 300µl of Isopropanol was added into each tube and 750µl of the mixture was transferred to a DNA mini column with a collection tube and centrifuged at 11,000rpm for a minute after which the flow through was discarded. This step was repeated to completely remove the mixture. A 500µl of DNA wash buffer was added to the column and centrifuged at 11,000rpm for a minute and then allowed to stand for 5mins to purify the DNA. The columns were centrifuged at 11,000rpm for a minute and flow through discarded. The columns were again centrifuged at 14,000rpm for 2mins to remove residual ethanol for optimal elution. A 25µl of deionized water was added to the column placed in a clean 1.5ml micro tube and placed inside block heat for a minute at 50°C. This was done to remove the DNA from the binding column. The columns were centrifuged at 11,000rpm for a minute to elute the DNA for Sequencing.

3.15 HPV DNA Sequencing Procedure

3.15.1 Principle:

Frederick Sanger created Sanger sequencing, also known as chain-termination sequencing, in 1977. This method relies on DNA template amplification using DNA polymerase and the inclusion of modified nucleotides, specifically dideoxynucleotides(ddNTPs), into the DNA fragment to be sequenced.A single-stranded DNA template, a DNA primer, a DNA polymerase, regular deoxynucleotidetriphosphates (dNTPs), and modified nucleotides (dideoxyNTPs) that stop DNA strand elongation are all required in the traditional chain-termination approach. The lack of a 3′-OH group in these chain-terminating nucleotides prevents DNA polymerase from forming a phosphodiester bond between two nucleotides, leading DNA polymerase to stop working when ddNTP is added.For detection in automated sequencing equipment, the ddNTPs would be radioactively or fluorescently labeled. The DNA sample is split into four independent sequencing processes, each of which contains the four typical deoxynucleotides (dATP, dGTP, dCTP, and dTTP) as well as DNA polymerase. Only one of the four dideoxynucleotides is introduced to each process (ddATP, ddGTP, ddCTP, or ddTTP).The resultant DNA fragments are heat denatured and sorted by size using gel electrophoresis after rounds of template DNA extension from the bound primer. A denaturing polyacrylamide-urea gel with each of the four reactions run in one of four distinct lanes is commonly used for this (lanes A, T, G, C).Autoradiography or UV light can then be used to visualize the DNA bands, and the DNA sequence can be read directly from the X-ray film or gel image.

3.15.2 Procedure

Primer for sequencing is Gp6+: 5′GAAAAATAAACTGTAAATCATATTC3′

Sequencing machine (Beckman Coultier CEQ 2000XL). Genotyping was achieved by direct sequencing using the Gp 6+ oligoprimer. The re-suspended samples were then overlaid with one drop of mineral oil from the kit and loaded on the sample plate into the instrument to start the process of sequencing.Sequencing was done using the Terminator version 3.1 cycle sequencing kit reaction (Applied Biosystems, Foster City, California, USA). All reagents were kept on ice while mixing the sequencing reagents; the sequencing reagent was prepared using 7µl of deionized water, 3µl of DNA template, 2µl of primer and 8µl of Dye Terminator Cycle Sequence DTCS Quick start master mix. This was inserted into the sequencing machine using the following conditions: 96°C for 20seconds, 50°C for 20 seconds and 60°C for 4 minutes in 30 cycles. The sequencer used was ABI PRISM 310 genetic analyzer by Applied Biosytems, Foster City, California, USA; Model 310 (Manga *et al.*, 2015).

3.16 Phylogenetic Analysis

3.16.1 Principle

Phylogenies are useful for addressing a variety of biological topics, including links between species or genes, the genesis and spread of viral infection, and population shifts and migration patterns. Phylogenetic analysis has reached new heights thanks to advances in sequencing technologies. Phylogenies have penetrated practically every discipline of biology, and an experimental biologist may feel overwhelmed by the plethora of phylogenetic methodologies and software packages presently available(Yang & Rannala, 2012).

3.16.2 Procedure

Sequence analysis of DNA samples was carried out to confirm the genotype of HPV. The Finch TV (version 1.4.0) software was used to edit chromatographs. All nucleotide sequences

obtained were screened using the online BLAST (Basic Local Alignment Search Tool) http://blast.ncbi.nlm.nih.gov/Blast.cgi to search for similarity between sequences and previously reported sequence in the database that are closely related. Standardisation and classification of HPV types was done in accordance with the international HPV reference center (www.hpvcenter.se) (de Villers *et al.,* 2004; Bernard, 2013). The following are the accession numbers of different HPV reference sequences used in this study;

Table 3.1: Human Papilloma Virus reference sequence

Accession numbers of different HPV reference sequence
JN617899.1, MH607475.1, LC155254.1, X94164.1, HQ834589.1, JQ902133.1, MK463926.1, JN617887.1, LC155235.1, DQ315392.1, MH028426.1, MK211166.1, KU707814.1, MF288657.1, KJ467243.1, KU550624.1, JN041072.1, JN617892.1, KU163584.1, KJ754580.1, KR674072.1, JN383600.1, AB601047.1, AB601048.1, JX912950.1, FJ797813.1, HQ834585.1, JN617895.1, MG195999.1, KU050127.1, EU911303.1, KR674075.1, KU961847.1, JN617890.1, EF140819.1, LC155223.1, GQ179959.1, DQ448184.1.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou& Nei, 1987). The optimal tree with the sum of branch length = 6.39480300 is shown and the percentage replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Evolutionary distances between the reference sequences and the isolated sequences were computed using the Maximum Composite Likelihood method (Tamura *et al.,* 2004) and are in the units of the number of base substitutions per site involving 60 nucleotide sequences. All ambiguous positions were removed for each sequence pair using pairwise deletion option. There was a total of 8112 positions in the final dataset with which evolutionary analyses were conducted in MEGA X (Kumar *et al.,* 2018).

3.16.1 Sequence alignments and Mutation analysis

The query sequence in FASTA format was analyzed using UniproUgene v33.0 which allows quick identification and characterization of the viral strain. The tool identifies mutations for the gene of query genome of Sequence 3 and Sequence 4 with respect to the reference sequences. Multiple sequence alignment was performed using CLUSTALW on UniproUgene software.

3.17 Data Analysis

All Data obtained from the questionnaire and results of test were statistically analysed for statistical association using Chi square (χ^2) at 95% confidence interval and 0.05 significance level. The analysis was performed using IBM Statistical Package for Social Sciences version 23 statistical software package.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Out of the 501 samples screened, 55 were found positive for HPV infection, representing 10.98%. The prevalence of HPV infection among women attending Asokoro District hospital was more (4.19%) when compared to other hospitals investigated (Table 4.1).

Study Area	**Number of Samples**	**Number positive for HPV**	**Percentage positive (%)**
Abaji	83	3	0.598
Asokoro	84	21	4.192
Bwari	83	2	0.399
Kuje	84	19	3.792
Kwali	83	5	0.998
Nyanya	84	5	0.998
Total	**501**	**55**	**10.98**

Table 4.1: Prevalence of HPV in the Federal Capital Territory

It was observed that women within the age group (25- 34 years) had more infection (4.80%) than other age groups (Table 4.2).

Table 4.2: Prevalence of HPV in relation to Age

Age group (years)	Sample screened	HPV positive	Prevalence (%)
15-24	21	1	0.20
25-34	204	18	3.60
35-44	192	24	4.80
45-54	73	8	1.60
55-64	11	4	0.80
Total	**501**	**55**	**10.98**

P- value 0.050 n = 501

It was observed that married women recorded more rate of infection (7.98%) when compared to the women that were single or widowed (table 4.3).

Table 4.3: Prevalence of HPV in relation to Marital status

Marital Status	**Sample screened**	**HPV positive**	**Prevalence (%)**
Single	49	8	1.60
Married	436	40	7.98
Divorced/ Widowed	20	7	1.40
Total	**501**	**55**	**10.98**

P- value 0.000 n = 501

Civil servants were found to be more infected (5.60%), followed by business women and the least rate of infection was found with women that were students (table 4.4).

Table 4.4: Prevalence of HPV in relation to Occupation

Occupation	**Sample screened**	**HPV positive**	**Prevalence (%)**
Civil servant	252	28	5.60
Student	21	1	0.20
House wife	67	5	1.00
Business	153	21	4.20
Farming	8	0	0.00
Total	**501**	**55**	**10.98**

P- value 0.427 n = 501

It was observed that women with tertiary education had more rate of infection (7.98%) followed by those with post primary education (2%) and the least was with women that had primary education qualification (table 4.5).

Table 4.5: Prevalence of HPV in relation to Educational Status

Educational status	**Screened sample**	**HPV positive**	**Prevalence (%)**
Primary	59	5	1.00
Secondary	92	10	2.00
Tertiary	350	40	7.98
Total	**501**	**55**	**10.98**

P- value 0.000 n = 501

Statistical analysis to determine the relationship between the rate of infection and the risk factors considered revealed that HIV status of the participant and number of sexual partners had significant relationship (table 4.6).

Table 4.6: Prevalence of HPV in relation to risk factors

S/N	Factors	Total Number (%)	HPV +VE Number (%)	HPV –VE Number (%)	P-value
1	**Use of Contraceptives**				
	Yes	49 (9.80)	3 (0.60)	43(9.20)	0.304*
	No	452 (90.2)	52 (10.40)	400(81.80)	
2	**History of sexually transmitted Infection**				
	Yes	200(39.90)	26 (5.20)	174(34.7)	0.238*
	No	301(60.10)	29 (5.80)	272(54.3)	
3	**Smoking**				
	Yes	5(1.00)	1 (0.20)	4(0.8)	0.399*
	No	496(99.00)	54 (10.78)	442(89.78)	
4	**HIV status**				
	Positive	64(13.00)	15 (3.00)	49(10.00)	0.001†
	Negative	431(87.00)	39 (7.8)	392(79.20)	
5	**Number of sexual Partner**				
	Single	479(95.60)	49 (9.80)	430 (85.8)	0.012†
	Multiple	22(4.40)	6 (1.20)	16(3.20)	
6	**No of children**				
	0 – 2	282 (56.30)	31 (6.20)	251(50.1)	0.466*
	3 – 5	193(38.50)	23 (4.60)	170(33.90)	
	6 and above	26(5.20)	1 (0.20)	25(5.00)	
7	**Complication History**				
	Miscarriage	143(28.50)	18 (3.60)	125(24.9)	0.635*
	Stillbirth	19(3.80)	2 (0.40)	17(3.40)	
	Both	31(6.20)	5 (1.00)	26(5.20)	
	None	308(61.50)	30 (6.00)	278(55.5)	
8	**Alcohol consumption**				
	No	371(74)	34 (6.80)	337(67.2)	0.078*
	Mild	80(16.00)	12 (2.40)	68(13.6)	
	Moderately	50(10.00)	9 (1.80)	41(8.20)	
	Heavy	0(0)	0 (0)	0(0)	
9	**Age at first sexual intercourse**				
	>18	105 (21.00)	11(2.20)	94(18.80)	0.853*
	<18	396(79.00)	44 (8.80)	352(7.20)	

† significant difference exists, * No significant difference.

Analysis of PCR Results/ HPV DNA detection

Human papilloma virus was detected in 15 samples out of the 55 samples (27.27%). **Plate I** shows the gel electrophoresis of HPV PCR amplicons. Lane 1, 12, 24, 37, 47, 48, 63, 67and100 shows a clear positive amplicon of 150bp. In **Plate II**, lane 293, 332, 351, 365, 381 and 394 also shows a positive result.

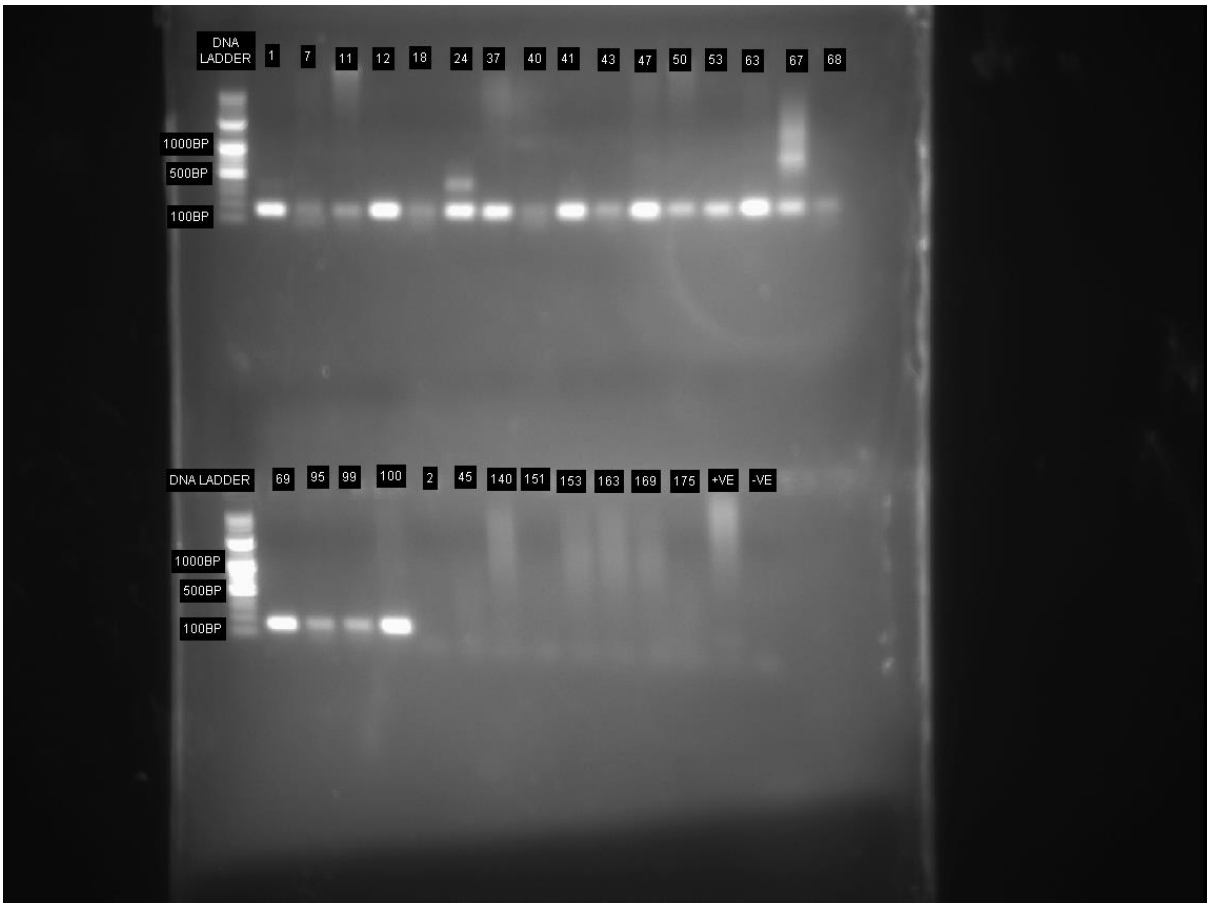


Plate I: Detection of HPV isolate by Gel electrophoresis using 1.5% Agarose Gel Stained with Ethidium Bromide. The first lane had the ladder followed by samples tested and lastly the negative (-ve; nuclease free water) and Positive (+ve) control

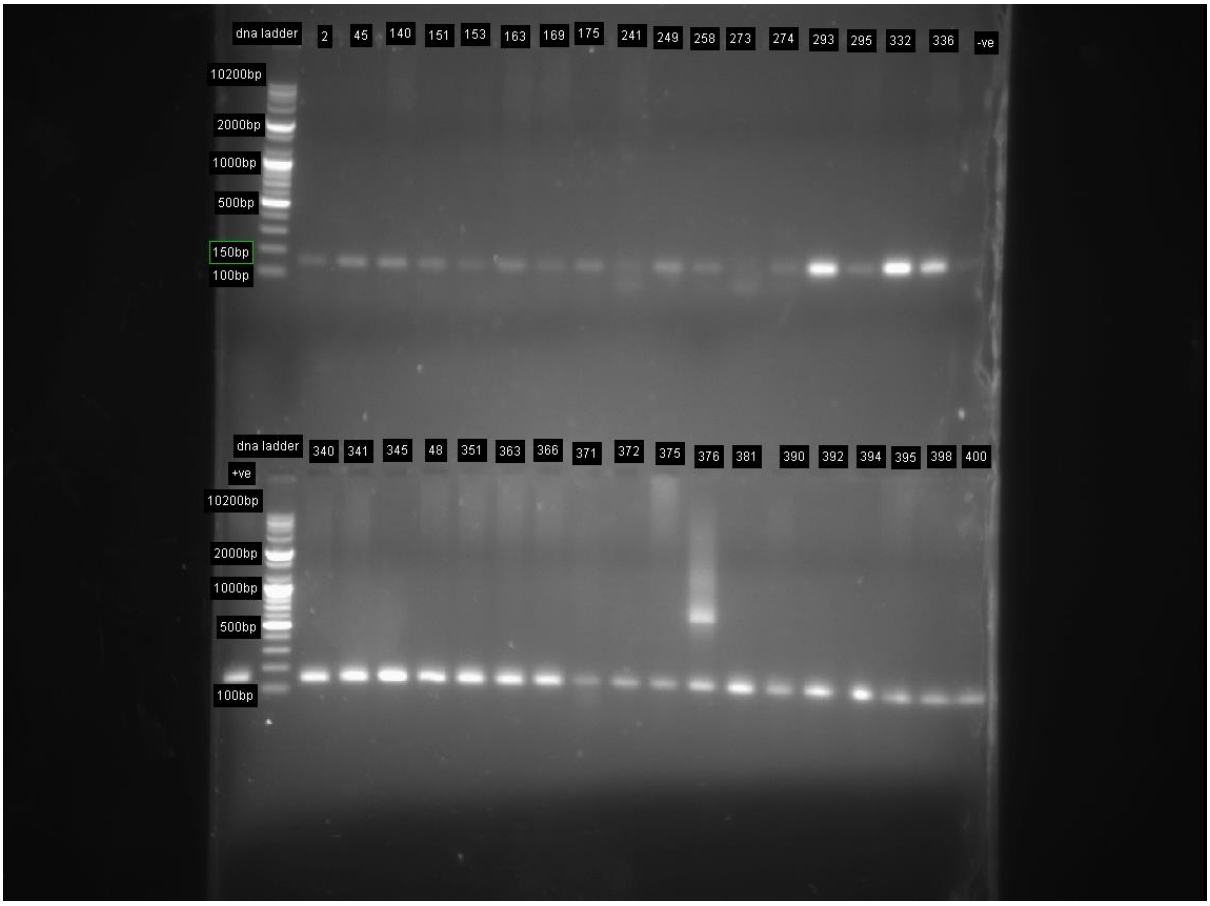


Plate II: Detection of HPV isolate by Gel electrophoresis using 1.5% Agarose Gel Stained with Ethidium Bromide. The first lane had the ladder followed by samples tested and lastly the negative (-ve; nuclease free water) and Positive (+ve) control

A total of fifteen samples were successfully sequenced and 9 HPV genotypes identified which are HPV- 6, 16, 18, 31, 58, 66, 70, 72 and 81. The result of HPV sequencing included high-risk HPV types such as HPV16 (13.33%), HPV18 (13.33%), HPV 31 (6.67%) and HPV 58 (13.33%). HPV 66which fall within the probable high- risk had a prevalence of 6.67%. Other HPV types identified in this study are characterised under the low- risk HPV types and they include: HPV 6 (6.67%), HPV 70 (26.67%), HPV 72 (6.67%) and HPV 81 (6.67%). Among all HPV types isolated, the most prevalent HPV type was HPV 70 (Table 4.7).

Table 4.7: Frequency of HPV types identified

Serial Number	**HPV Type**	**Frequency**
1	HPV- 18	2 (13.33%)
2	HPV- 70	4 (26.67%)
3	HPV- 6	1 (6.67%)
4	HPV- 72	1 (6.67%)
5	HPV- 31	1 (6.67%)
6	HPV- 81	1 (6.67%)
7	HPV- 16	2 (13.33%)
8	HPV- 58	2 (13.33%)
9	HPV- 66	1 (6.67%)

Table 4.8: BLAST N Pairwise Alignment of Fifteen Amplicon Sequenced against reference strains

Sample	Query cover	Length	Score	E-Value	Identities	Reference Strain	Inference
1	100	99	172	2e-39	98%	MK211166.1	HPV- 18
12	97	108	141	5e- 40	97.59%	JX912950.1	HPV- 70
24	99	103	174	5e- 40	97.12%	MG195999.1	HPV- 18
37	100	91	150	7e- 33	95.79%	KR674075.1	HPV- 6
47	100	95	135	2e- 28	91.92%	JQ902133.1	HPV- 72
48	97	89	75.2	3e- 10	83.33%	JN617892.1	HPV- 31
63	99	102	154	6e- 34	93.52%	JN617895.1	HPV- 70
67	97	88	86.9	1e- 14	86.73%	LC155235.1	HPV- 81
100	92	95	148	3e- 32	96.70%	EF140819.1	HPV- 16
293	88	84	95.1	3e- 16	92.21%	DQ448184.1	HPV- 16
332	53	98	86.1	2e- 13	96.23%	JN383600.1	HPV- 70
351	98	74	86.9	1e- 13	88.31%	KU550624.1	HPV- 58
365	98	70	61.7	5e- 06	81.82%	JN041072.1	HPV- 58
381	100	88	125	2e- 25	92.39%	JN617895.1	HPV- 70
394	53	73	54.5	8e- 04	92.68%	JN617899.1	HPV- 66

Phylogenetic Analysis result

The phylogenetic tree was constructed using 15 isolates from this study and 45 reference strains selected from NCBI data based on percentage similarity, the tree had 4 clades. Clade 1 had most of the reference strains and had a branch length of 100% (Figure 3).

The two HPV 16 strains isolated in this study were in Clade 2 on the phylogenetic tree. Both strains had the same ancestral parents with a branch length of 92 indicating a high similarity between the two HPV 16 strains.

Clade 3 had a branch length of 68 with the two HPV 58 strains isolated in this studyand they both shared same ancestral parents.

The fourth clade had HPV 18 strains, HPV 81, HPV 72, HPV 6, HPV 31, HPV 70 and reference strain MG195999.1 and GQ179959.1 with a branch length of 75 indicating the level of relatedness. From the phylogenetic tree, HPV 6 was found to be closely related to reference strain GQ179959.1 isolated from Iran with a branch length of 67% while HPV 18 was found to be closely related to the reference strain MG195999.1 isolated in Bayelsa Nigeria with a branch length of 89 indicating a high percentage similarity between the two strains (Figure 3).

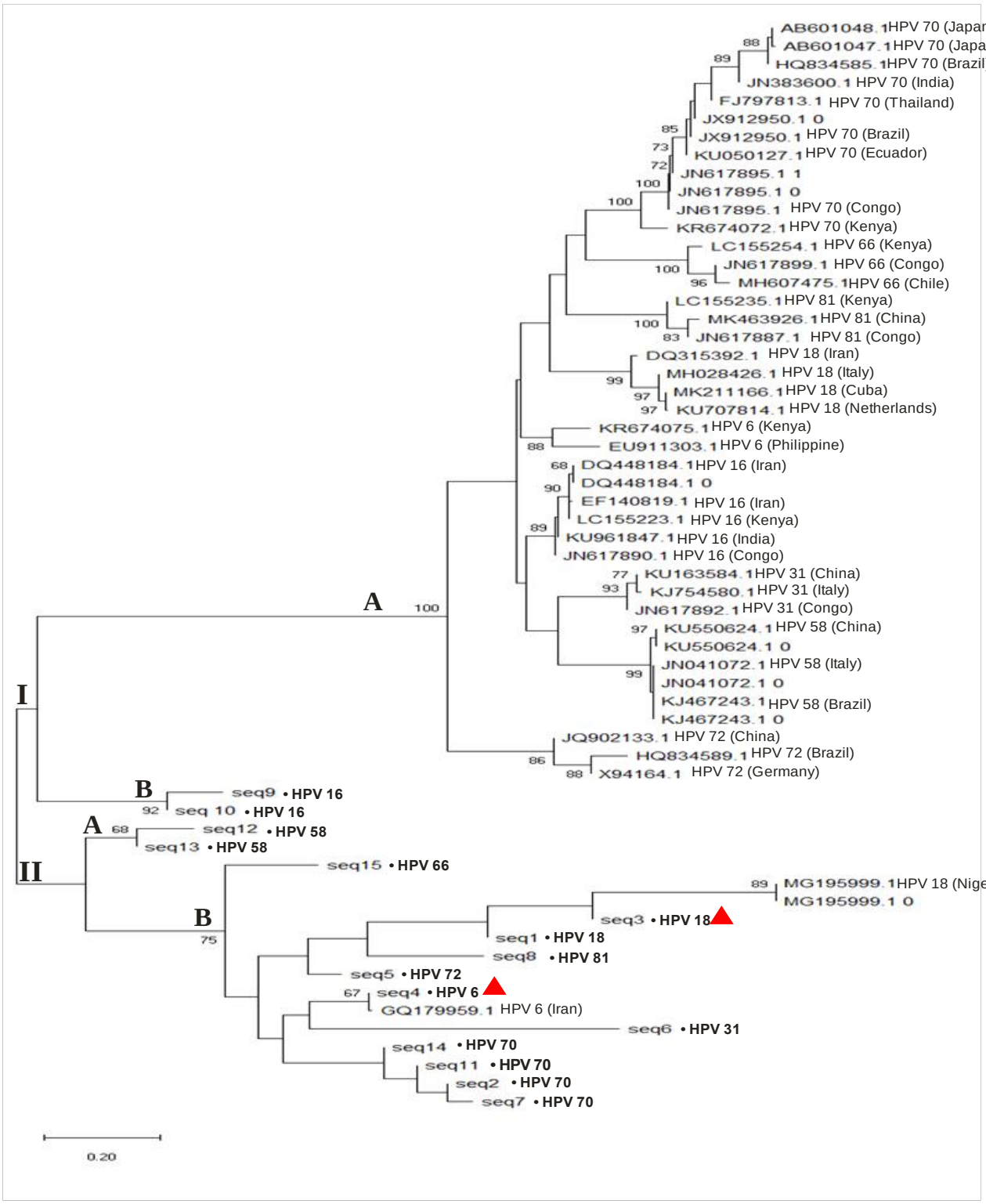


Figure 4.1: Bootstrapped Neighbour-Joining Phylogenetic Tree

From the variation analysis between HPV 6 isolated in this study and the reference strain GQ179959.1 it was found that both strains differed by Intercalary deletion mutation at position 101, 102, 120 and 133 of the sequence (figure 4). Intercalary deletion mutation is a deletion of nucleotide sequences that occurs from the interior of a gene (Lewis, 2004) as a result from unequal crossing over or breaking without rejoining during DNA replication. Therefore, there was no sequence dissimilarity between HPV 6 and GQ179959.1.

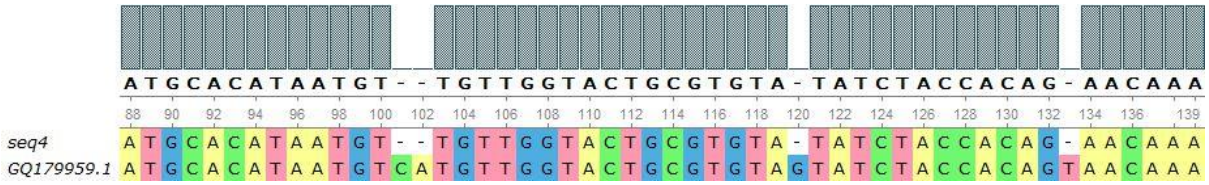


Figure 4.2: MSA showing point mutations between sequence 4 and reference strain

HPV 18 in addition to deletion mutation at position 263 and 304 experienced a base substitution mutation or point mutation with respect to the reference sequence. The point mutation occurred at position 336, where Guanine is replaced with Adenine, and position 357, where Thymine is replaced with Adenine (Figure 5). Therefore, there were two sequence dissimilarities between HPV 18 and MG195999.1.

Figure 4.3: MSA showing substitution and deletion mutations between sequence 3 and reference strain.

4.1 DISCUSSION

The study investigated the prevalence of HPV infection among women attending selected hospitals in the Federal Capital Territory, Nigeria. The overall prevalence of HPV in the study area was 10.98%. The prevalence in this study is lowwhen compared to the prevalence (18.6%) reported by Nejo *et al.* (2018) in a study conducted in Southwest Nigeria. The low prevalence in this study could be attributed to a variety of variables, including the method and sensitivity of the HPV test employed, as well as variances in research populations with differential exposures to different risk factors due to socio-cultural differences. The prevalence in this study was also low when compared with the prevalence (21.6%) reported by Schnatz *et al.* (2008) in a studyconducted in Okene Kogi state, Nigeria. The low prevalence recorded in this study could be attributed to high level of awareness in the study area as the Federal Capital territory and has a population of educated and informed people from different backgrounds across the country who have a general knowledge about HPV as a result of public awareness programmes organised from time to time by government and non-governmental organizations to inform the population. A study also from the north central Nigeria reported a higher prevalence of 37% (Akarolo-Anthony *et al.*2014). A prevalence rate of 48.1% was reported by Manga *et al.* 2015 in Gombe the North-eastern part of Nigeria and a higher prevalence rate of 70% was also recorded in a study by Auwal *et al.* (2014) in Kano state.The high level of infection within the North might be due to variation among the study population with varying exposure to different risk factors, diverse culture and geographical location, also owing to the fact that the Northern part of Nigeria is known for polygamous family lifestyle as reported by Manga *et al.*(2015).

Previous studies conducted in the Sub-Saharan Africa indicated a generally high prevalence rate with some variations depending on how the target group was selected and also the method of assay used. Using a PCR based assay, HPV prevalence rate was 40% in

Mozambique (Castellsague *et al.*, 2001), 31% in Harare, Zimbabwe (Gravitt *et al.*, 2002), 18% in Dakar, Senegal (Xi *et al.*, 2003) and 44% in Nairobi, Kenya (DeVuyst *et al.*, 2003). A prevalence rate of 66.1% in Burkina Faso (Didelot- Rousseau *et al.*,2006) and 60.7% in Sudan (Salih *et al.*,2010) was also recorded. Using the Hybrid Capture assay, 17% HPV prevalence was reported in rural Uganda (Serwadda *et al.*, 1999).

The distribution of HPV infection within the study areas, Asokoro District hospital had more prevalence of 4.19% as shown in Table 4.1 and this could be attributed to the fact that the hospital serves as a reference centre for HIV positive cases and HPV screening centre within the federal capital territory. This agrees with a study by Gillison, (2001) who reported that the probability of HPV infection is increased by about 100% in HIV infected persons.

The age pattern showed peak of HPV positivity in women within the age range of 35-44 years (4.8%). Similar study by Fadahunsi *et al.* (2013) has reported high rate of infection with HPV among women within the age group 35- 44years. It is assumed that since cervical cancer is more common in women older than 35 years (Adam *et al.*, 2000), suggesting that HPV infection occurs at a younger age and then gradually persist over a period of time. The high incidence of HPV infection among women within the age group 35-44 years could be attributed to decline in the strength of the immune system. Middle age women who remain persistent carriers of HPV are now considered the high-risk group for cervical cancer (Herrero *et al.*, 2000).Prevalence in relation with age group is also similar to finding by Herrero *et al.* (2000) who reported a high prevalence among women within 35-54 years in Costa Rica and a study by Lazcano-Ponce *et al.*, (2001) who also reported a high prevalence rate among 35-44 years in Mexico. Statistically, there was a significant difference between HPV infection and the different age groups (P-0.050). The variation in prevalence of HPV in relation to age is well documented and appears to largely reflect differences in sexual behaviour across geographical regions (Smith *et al.*, 2008).

The highest prevalence of HPV infection was found among women were married. This is linked with the assumption that morally, sexual activity of a woman is supposed to be at its peak when married. This may be an explanation for the high level of HPV infection found among this group of individuals in this study. This agrees with a study by Akarolo-Anthony *et al*. (2014) who reported a higher prevalence of HPV among the married (61%) over the unmarried (39%). A higher positivity rate among singles than married was reported by Thomas *et al*. (2004).Statistical analysis in this study indicated a significant relationship between the rate of HPV infection and marital status.

The rate of HPV infection was higher among women who were civil servants when compared to other form of occupation. Statistical analysis indicated that no significant relationship was found between HPV infection and occupation of women. This agrees with studies reported by Baloch *et al*.(2016) and Traore *et al*.(2016).

This study reported a high prevalence of HPV infection among women with tertiary form of education which is similar to a study by Manga *et al.* (2015) who recorded a significant relationship between the rate of HPV infection and the level of education. This study however contradicts the study by Esere, (2008) who found that lack of formal education is associated with the high- risk sexual practices resulting in the increased presence of sexually transmitted infections like HPV.

This study showed a significant relationship between the HPV infection and HIV status agreeing with the study by Vem *et al.* (2013) in Jos who recorded the HPV prevalence of9.0% among HIV positive women. The rate of infection in this study could be attributed to the fact that HIV is known to compromise the immune system of an individual giving room for persistence of HPV infection.

This study recorded a high rate of HPV infection among women who had between 0 to 2 number of children. This is similar to a study by Azua *et al.* (2015) who recorded a higher infectionrate among women who hadtwo or more number of children. This may be attributed to hormonal changes in pregnancy resulting in reduced immunity and also the exposure of the ectocervix during repeated child birth resulting in easy attachment and proliferation of the virus. However, it was also observed in a study by Fadahunsi *et al.*(2013) and Nyengidiki *et al.* (2016) that women with high parity (three pregnancies or more) accounted for 83.7% of women with high risk HPV. There was no significant relationship between the rate of HPV infection and number of children (parity) in this study. This is in line with Thomas *et al.* (2004) and Sarma *et al.*(2013). However, CDC (2015) and Cancer research UK (2013) also stipulated the women who have had more children had double risk of HPV infection. This is an indication that the risk of getting infected with the virus in relation to the number of times a woman gives birth doubles due to the occurrence of cervical trauma at the time of delivery. There are also tendencies of weakening and rupturing of epithelial cells around the cervix thereby enhancing chances of infection when in contact with the virus.

The study indicated that there was also a significant relationship between the HPV infection rate and the number of sexual partners (P= 0.012). This is similar to study by Nascimento *et al.* (2018) in Brazil and Manga *et al.* (2015) in Nigeria which recorded a high prevalence among women who had multiple sexual partners.The presence of multiple sexual partners increases the risk of acquisition of sexually transmitted infections like HPV.Also a similar research work done in Guinea by Keita *et al.,* (2009) also recorded a high prevalence of HPV infection among women with multiple number of sexual partners. He also indicated that the influence of sexual orientation of women was a contributory factor to the occurrence of HPV. The role of multiple sexual partners in acquiring HPV infection was also observed in a study done in Columbia by Munoz *et al., (*2004). The presence of multiple sexual partners increases

the risk of acquisition of sexually transmitted infections. Data supporting sexual intercourse as the principal route of genital HPV infection (Partridge & Koutsky, 2006), as well as higher risk of HPV acquisition from new and recent sexual partners (Winer & Koutsky, 2004), have all contributed to this conclusion.

Smoking was found not to be associated with the rate of HPV infection in this study. This could be attributed to the fact that cultural and moral beliefs in the study area frown at smoking habits in women, which may have contributed to the low number of smokers in this study. However, similar study by Jensen *et al.*(2013) had established a link between heavy smoking and persistence of oncogenic strains of HPV. This was attributed to the nicotinic inhibition of the phagocytic property of cervical macrophages resulting in the persistence of the virus.Studies have shown that exposure to tobacco may affect the ability of the host to mount an effective local immune response against viral infections, as it has been shown that smoking may reduce the number of Langerhans cells and other markers of immune function (Poppe *et al.,* 1995).

There was no significant association between the rate of HPV infection and oral contraceptive use in this study but a study by Moreno *et al.* (2002) reported otherwise and a possible explanation is that longer exposure to oral contraceptive for more than 5 years can pose high risk of infection with HPV. This is as a result of change in hormonal imbalance which gives room for the proliferation and persistence of HPV infection.

In this study, HPV positive was found to be high at a young age (<18 years) during the first sexual intercourse. This may be due to the fact that earlier intercourse exposes young individuals to other dangerous sexual behaviours, such as having more lifetime sexual partners and concurrent relationships (Aral & Holmes, 2008). The prevalence of HPV infection increases with increasing numbers of life time sexual partners, which is inagreement

with previous findings by Thomas *et al.* (2004). There was no statistical relationship recorded.

The HPV DNA detection using PCR was done and 15 samples out of the total of 55 samples were successfully sequenced, giving a frequency of 27.27% (15/55). The frequency of HPV Types (27.27%) in this study is lower than the study in Abuja by Akarolo- Anthony *et al.* (2014) who had a frequency of 37%. This might be attributed to the fact that different method of HPV assay and different study population used. Studies by Nejo *et al.* (2019), Dareng *et al.* (2016) recorded prevalence of 17.3% and 23.7% respectively. Studies reported by Ayse *et al.* (2019) among Turkish women and Aziz *et al.* (2018) among Pakistani women were 6.5% and 4.7% respectively. The reason for the low prevalence rate could be as a result of the customary monogamous behaviour within the society of Turkey and Pakistan. Bedoya-Pilozo *et al.* (2018) however recorded a high prevalence rate of 68.1% among women in Ecuador.

A total of 9 HPV types were detected (HPV- 6, 16, 18, 31, 58, 66, 70, 72 and 81). The most prevalent of HPV type recorded in this study was HPV- 70. This study is in agreement with studies by Aziz *et al.* (2018) who also reported a high prevalence of HPV- 70.Studies conducted in Nigeria (Fadahunsi *et al.*, 2013, Nweke *et al.*, 2013, Adegbesan-Omalibu *et al.*, 2014 and Nejo *et al.*, 2019) in Southwestern, Nigeria, reported predominant HPV type isolated to be HPV-31. The studies they carried out indicated high prevalence among high risk type as compared to this study that had a high prevalence among Low risk HPV types. This may be due to the geographic location of study area and also the type of assay used.

The high- risk types found were HPV 16, 18 and 58. This is similar to studies recorded by Manga *et al.* (2015) andAuwal *et al.* (2014) where HPV-16 and 18 were the most prevalent. This might imply that there is a persistent of similar HPV types within the Northern

geographic region of Nigeria which is close in proximity to the study area.This study has significance as various studied have indicated the role of HPV 16 and 18 in cervical cancer development, this then suggest that there should be strong advocacy for the use of HPV DNA typing technique in routine diagnosis of HPV infection and cervical cancer. Also, the isolation of high- risk HPV among healthy women has reiterated the fact that in most cases, there is almost no clinical signs and symptoms of HPV infection in the individual, therefore the need for routine HPV screening.

This study is also similar to a study in West African region with type 16 and 18 been the most common high- risk HPV types isolated (Munoz *et al.,* 2002). Studies among Sub- Saharan Africa women showed that HPV- 16, 18, 33, 35, 45 and 52 are the most commonly detected types (Denny *et al.,* 2014) and also responsible for 80% cancers (Galloway, 1998). Bedoya-Pilozo *et al* (2018) also identified high risk HPV- 16 and 58 as the most common type isolated among women in Ecuador.

This study identified a woman with genital warts who had low- risk HPV- 81 and this is similar to a study by Nejo *et al.* (2019) who reported same. This implies that low risk HPV may be associated with genital warts and so relying on presence or visibility of warts alone is not enough as adequate HPV DNA testing will be required to identify the type.

The phylogenetic tree has shown that HPV 6 isolated in this study was closely related to reference strain GQ179959.1 (HPV 6) isolated from tissue sample of cervicitis in an Iranian woman by Mirzaie- Kashani *et al*. (2014). The similarity is the fact that both strains were isolated from women with same social behaviour of one partner throughout their marriage. This agrees with a study by Vaccarella *et al*. (2010) who reported that marital status played a role in an increased risk of HPV positivity.

HPV 6 have been classified as low risk HPV and cause genital warts but an infection could also be asymptomaticand persist for a while (Mirzaie-Kashani *et al.,* 2014). This justifies why the patient isolated in this study was a healthy individual since HPV infection can be asymptomatic and persistent. Majority of genital warts are associated with HPV 6, this affects the cervix causing inflammation of the cervix know as cervicitis (Cubie, 2013), this could be the reason why reference strain HPV 6 was isolated from samples of cervicitis tissues among the Iranian women.

The reference strain HPV- 6 had 139 nucleotide base pair, while the study strain had 91 base pair. Both strains were from the same ancestral origin with a branch distance of 67% indicating percentage similarity and had no hamming dissimilarity among the sequences after subjecting to variation analysis. But it was found that HPV- 6 in this study differed from reference strain HPV- 6 by Intercalary deletion mutation at position 101, 102, 120 and 133 of the sequence (Figure 4.) which could be the possible cause of variation on number of nucleotide base pairs.

There was a close relationship on the phylogenetic tree between HPV- 18 isolated in this study to reference strain MG195999.1 isolated in Bayelsa Nigeria. Both strains were isolated from the cervix of asymptomatic women. This supports a study that indicated that HPV- 18 African (Af) variants showed more similarity to one another than non-African variant. A study by Bruni *et al*. (2016) also reported a high prevalence of HPV- 18 among women with normal cytology across the world and was consistently among the most common HPV type. HPV 18 has been classified as high risk, therefore Nejo *et al*.(2019) reported in study in Ibadan Nigeria that high risk HPV- 18 is found in healthy individuals and this buttress the fact that most HPV infections don't manifest clinically.The variation analysis indicated there were two sequences dissimilarity between HPV 18 in this study and reference strain MG195999.1 (Table 11). However, HPV 18 in addition to deletion mutation at position 263

and 304 experienced a base substitution mutation or point mutation with respect to the reference sequence. The point mutation occurred at position 336, where Guanine is replaced with Adenine, and position 357, where Thymine is replaced with Adenine (Figure 5). This agrees with studies by Condo- Ferraez *et al.* (2017), Ramas *et al.* (2018), Zhang *et al.* (2018) and Cui *et al.* (2018) that distribution of HPV types to an extent is not affected by geographical distancebut emphasizes on the of nucleotide polymorphism and mutational changes in viral genes. The variation analysis also supports a study by Bernard *et al.* (2010) who indicated that disparity in HPV strains could be as result of mutation either by point mutation, deletion and insertion. Another factor causing variation of both HPV- 18 in this study and reference strain MG195999.1 could be as result of difference in the genetic makeup of the women from whom the virus was isolated as Xi *et al.* (2006)reported in a study that genetic makeup of host causes disparity in HPV strains isolated.A difference was also observed the base pairs of HPV- 18 (103) in this study and the reference strain MG195999.1(400) base pairs.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The prevalence of HPV infection in the study area was found to be 10.8 %. Age group, Educational Status, Marital Status, Number of sexual partners and HIV status were the significant risk factors associated with cervical HPV infection in the study area. About 9 different HPV types (HPV- 6, 16, 18, 31, 58, 66, 70, 72 and 81) were identified with the most predominant type being HPV 70. Phylogenetic analysis revealed HPV- 6 relationship to a reference strain from Iran and also HPV- 18 relationship to a reference strain isolated for Bayelsa, Nigeria. Mutation analysis also found the occurrence of mutation in HPV- 6 and HPV- 18.

5.2 Recommendations

The prevalence of the HPV genotypes as reported in this study exposes the level of the burden of HPV infection in the study area.

i. There is therefore the need to increase the level of screening, since oncogenic strains with a high tendency to transform into malignancy were observed in this study.
ii. There is also a need to encourage Government agencies to promote a more inclusive HPV vaccine in their national immunization scheme which has a wider coverage as against the one currently available in the country. This should be administered to girls at the appropriate age if HPV transmission is to be reduced.
iii. There is also need for sexual behaviour education and awareness in order to reduce the impact of the risk factors identified in this study.

iv. Population based study need to be done for further characterization of HPV genotypes in our environment.

v. There should be strong advocacy for the use of HPV DNA typing technique in routine diagnosis of HPV infection and cervical cancer.

vi. The isolation of high- risk HPV among healthy women has reiterated the fact that in most cases, there is almost no clinical manifestation of HPV infection in the individual therefore the need also for routine HPV screening.

5.3 Contribution to knowledge

Results obtained from this study aside from creating awareness will enable the government and other interested non-governmental organizations to implement clinical and health policy interventions to control cervical cancer and other cancers associated with HPV among human population in the study area.

Furthermore, identification of strains within the study area will give room in future for the development of a candidate vaccine. Phylogenetic analysis will help identify the evolutionary history of different strains isolated and their common relationship.

REFERENCES

Abumere, S.I. (1984). The Future Population of the Federal Capital Territory, Abuja.*The Nigerian Journal of Economic and Social Studies*, 26 (3), 287-313.

Adam, E., Berkova, Z., Daxnerova, Z., Icenogle, J., Reeves, W.C., & Kaufman, R.H. **(**2000). Papillomavirus detection: demographic and behavioural characteristics influencing the identification of cervical disease. *American Journal of Obstetric Gynaecology*, 182**,** 257-264.

Adegbesan-Omilabu, M. A., Okunade, K. S., Omilabu, S. A. (2014). Oncogenic human papilloma virus infection among women attending the cytology clinic of a tertiary hospital in Lagos, South-West Nigeria. *International Journal of Research in Medical Sciences*, 2 (2), 625-630.

Akarolo-Anthony, S.N., Famooto, A.O., Dareng, E.O., Olaniyan, O.B., Offiong, R., Wheeler, C.M. & Adebamowo, C.A. (2014). Age-specific prevalence of human papilloma virus infection among Nigerian women. *BioMedCentral Public Health*, 14:656.doi: 10.1186/1471-2458-14-656.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. (1990).Basic local alignment search tool. *Journal of Molecular Biology*, 215,403–410.

Anorlu, R.I., Igwilo C.I., Akanmu A.S., Banjo A.A., Odunukwe N.N., Okany C.C., *et al*. (2007). Prevalence of abnormal cervical smears among patients with HIV in Lagos, Nigeria. *West African Journal of Medicine*,26(2), 143–7.

Anttila, T., Saikku, P., Koskela, P., Bloigu, A., Dillner, J. & Ikaheimo, I. (2001). Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. *Journal of the American Medical Association*, 285 (1), 47-51.

Aral, S.O. & Holmes, K.K. (2008). The epidemiology of STIs and their social and behavioural determinants: industrialized and developing countries. In: Holmes KK, Sparling PF, Stamm WE, Piot P, Wasserheit JN, Corey L, Cohen MS, Watts DH, editors. *Sexually Transmitted Diseases*, 4th. New York: McGraw-Hill, pp. 53–92.

Autier, P., Coibion, M., Huet, F & Grivegnee, A.R. (1996). Transformation zone location and intraepithelial neoplasia of the cervix uteri.*British Journal of Cancer*,74, 488-490.

Auwal, I.K., Aminu, M., Atanda, A.T., Tukur, J.& Sarkinfada, F. (2014). Prevalence and RiskFactors of High-Risk Human Papillomavirus Infections among Women Attending Gynaecology Clinics in Kano, Northern Nigeria. *Bayero Journal of Pure Applied Science*,6, 67–71.

Avci, G.A., Bozdayi, G., Taskiran, C., Ozkan, S. & Onan, M.A. (2013). Phylogenetic Analysis and Prevalence of Human Papillomavirus (HPV) in Women with several Cervical Pathologies. *Journal of Turkish Societyof Obstetrics and Gynecology*, 10 (3), 151-159.

Ayse Erdem Y., Berrin G. K., Ayse A. & Osman A. (2019). Investigation of human papillomavirus prevalence inmarried women and molecular characterization and

phylogenetic analysis of the virus.*Obstetrics and Gynecology Science* 62(4), 264-272.

Aziz, H., Huma, I., Humera, M., Shazia, F., Mohammad, F., Areej, A., Sobia, T., Sanum,N., Syeda, B. & Nuzhat, R. (2018). Human papillomavirus infection in females with normal cervical cytology: Genotyping and phylogenetic analysis among women in Punjab, *Pakistan International Journal of Infectious Diseases,* 66,83–89.

Azua T.E., Orkaa, P.Y. & Ega, R.A.I. (2015). Prevalence of Human Papilloma Virus (HPV) Infection among Pregnant Women attending Antenatal Care at General Hospital, Minna-Nigeria. *Journal Microbiology and Biotechnology Research*, 5(3), 38-44.

Baloch, Z., Yuan, T., Yindi, S., Feng, Y., Tai, W., Liu, Y., Liu, L., Zhang, A., Wang, B., Wu, X. & Xia X. (2016). Prevalence of genital human papillomavirus among rural and urban populations in southern Yunnan province, China.*Brazilian Journal Medical and Biological Research,* 49(6),e5254.

Bedoya-Pilozoa, C.H., Lex, G. M. M., Maylen, E., Sáncheza, M., Parrales, V. J. V., Denisse, M., Ibarraa, M.A., Quimis-Poncea, M., Karool, E. and Maciasa, K.E.(2018). Molecular epidemiology and phylogenetic analysis of human papillomavirus infection in women with cervical lesions and cancer from the coastal region of Ecuador. *Revista Argentina de Microbiologia.*50(2), 136-146.

Bernard, H. (2014). Taxonomy and phylogeny of papillomaviruses: An overview and recent developments. *Infection, Genetics and Evolution,* 18, 357-361

Bernard, H.U., Burk, R.D., Chen, Z., van Doorslaer, K., zur Hausen, H. & de Villiers, E.M. (2010). Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology,* 401,70-79.

Bontkes, H. J., deGruijl, T.D., Walboomers, J.M., Schiller, J.T., Dillner, J., Helmerhorst, T.J., Vereheijen, R.H., Scheper, R.J., & Meijer, C.J. (1999). Immune responses against human papillomavirus (HPV) type 16 virus-like particles in a cohort study of women with cervical intraepithelial neoplasia. II. Systemic but not local IgA responses correlate with clearance of HPV-16. *Journal of General Virology,* 80**,** 409-417.

Bosch, F.X., Burchell, A.N., Schiffman, M., Giuliano, A.R., de Sanjose, S. &Brun,i L. (2008).Epidemiology and natural history of human papillomavirus infections and type- specific implications in cervical neoplasia.*Vaccine,* 26 (10), 1-16.

Bosch, F.X., Lorincz, A., Munoz, N., Meijer, C.J. & Shah, K.V. (2002). The causal relation between human papillomavirus and cervical cancer. *Journal of Clinical Pathology,* 55 (4), 244- 265.

Bosch, F.X., Manos, M. M., Munoz, N., Sherman, M., Jansen, A. M., Peto, J., Schiffman, M. H., Moreno, V., Kurman, R., Shah, K. V. & International Biological Study on Cervical Cancer (IBSCC) Study Group. **(**1995). Prevalence of human papilloma virus in cervical cancer: a worldwide perspective. *Journal of National Cancer Institute.*87**,** 796-802.

Brooks, G., Carroll, C.K., Butel, J. & Morse, S. (2007). *Jawetz, Mclnick, &Adelberg's Medical Microbiology,* (24th Ed.), McGraw-Hill Companies, Inc., US, 597-600.

Bruni, L., Barrionuevo-Rosas, L., Alberto, G., Serrano, B., Mena, M., Gomez, D., Munoz, J., Bosch, F.X. & de Sanjose, S. (2016). Human Papillomavirus and Related diseases in Nigeria.*ICO Information Centre on HPV and Cancer (HPV Information Centre).* Summary Report.

Bruni, L., Diaz, M., Castellsagué, M., Ferrer, E., Bosch, F.X. & de Sanjosé, S. (2010). Cervical Human Papillomavirus Prevalence in 5 Continents: Meta-Analysis of 1Million Women with Normal Cytological Findings. *Journal of Infectious Diseases,* 202, 1789– 99.

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

Calore, E. E., Pereira, S.M.M. & Cavaliere, M.J. **(**2001). Progression of cervical lesions in HIV-seropositive women: a cytological study. *Diagnostic Cytopathology,* 24**,** 117-119.

Castellsague, X.& Munoz, N. (2003). Cofactors in Human Papillomavirus carcinogenesis-role of parity, oral contraceptives and tobacco smoking. *Journal of National Cancer Institute Monographs,* 31, 20-28.

Castellsague, X., Bosch, F.X. & Munoz, N. (2002). Environmental co-factors in HPV carcinogenesis. *Virus Research,* 89, 191- 199.

Castellsagué, X., Menéndez, C., Loscertales, M. P., Kornegay, J. R., dos Santos, F., Gómez-Olivé, F. X., Lloveras, B., Abarca, N., Vaz, N., Barreto, A., Bosch, F.X. &Alonso, P. (2001). Human papillomavirus genotypes in rural Mozambique. *Lancet,* 358(9291):1429-30PMID: 11705494, DOI: 10.1016/S0140-6736(01)06523-0.

Castro, A. F., Dominguez, A., Pusche, K., Van De Wyngard, V., Snijders, P. J. F., Franceschi, S., Pawlita, M. & Ferreccio, C. (2014). Serological prevalence and persistence of high-risk human papillo infection among women in Santiago, Chile. *BioMedCentral Infectious Diseases,* 14:361.

Center for Disease Control and Prevention. (2013). STD curriculum for clinical educators. *Genital Human papillomavirus.* 1–37.

Center for Disease Control and Prevention (2008).Genital HPV Infection – CDC Fact Sheet.*http://www.cdc.gov/std/HPV/STDFact-HPV.htm.*

Chang, Y. E. & Laimins, L. A.(2000). Microarray analysis identifies interferon-inducible genes and Stat-1 as major transcriptional targets of human papillomavirus type 31. *Journal of Virology,* 74**,** 4174-4182.

Chan, P. K., Chan, M.Y., Li, W.W., Chan, D.P., Cheung, J.L. & Cheung, A.F. (2001). Association of human beta-herpesviruses with the development of cervical cancer: bystanders or cofactors? *Journal of Clinical Pathology,* 54**,** 48-53.

Chesson, H.W., Dunne, E.F., Hariri, S. & Markowitz, L.E. (2014).The estimated lifetime probability of acquiring human papillomavirus in the United States. *Sexually Transmitted Diseases,*41(11), 660-664.

Choi, Y. J & Park, J. S. (2016).Clinical significance of humanpapillomavirus genotyping. *Journalof Gynecologic Oncology,*27(2), e21doi: 10.3802/jgo.2016.27.e21.

Conde-Ferraez, L, Pacheco-Arjona, R., Novelo Canul, C., Gomez-Carballo, J., Ramirez-Prado, J. H.,Ayora-Talavera, G. &González-Losa, M.R. (2017). Genetic variability in E6 and E7 oncogenes from human papillomavirus type 58 in Mexican women. *Intervirology,*60, 235-46.

Cubie, H. A. (2013). Diseases associated with human papillomavirus infection *Virology,* (445), 21-34.

Cubie, H.A., Seagar, A.L., McGoogan, E., Whitehead, J., Brass, A. & Arends, M.J. (2001). Rapid real-time PCR to distinguish between human papillomavirus types 16 and 18. *Journal of Clinical Pathology Molecular Pathology,* 54,24–29.

Cubie, H. A., Seagar, A.L., Beattie, G.J, Monaghan, S., & Williams, A.R.W. (2000). A longitudinal study if HPV detection and cervical pathology in HIV infected women. *Sexually Transmitted Infections*,76, 257-261.

Cui, F, Zhang, Z, Xu, J, Ding, X, Mu, X. & Wan, Q. (2018). Genetic variability and lineage phylogeny of human papillomavirus type 45 based on E6 and E7 genes in Southwest China. *Virus Research*255, 85-9.

Dareng, E. O., Famooto, A. O., Akarolo-Anthony, S.N., Offiong, R.A., Olaniyan, O., Dakum, P.S., Wheeler, C.M, Fadrosh, D., Yang, H., Gajer, P., Brotman, R.M., Ravel, J. & Adebamowo, C.A.(2016). Prevalent high-risk HPV infection and vaginal microbiota inNigerian women. *Epidemiology and Infection,* 144, 123–137.

Das, B.C., Gopalkrishna, V., Hedau, S. & Katiyar, S. (2000). Cancer of the uterine cervix and human papillomavirus infection. *Current Science,* 78(1), 10.

Denny, L., Adewole, I., Anorlu, R., Dreyer, G., Moodley, M., Smith, T., Snyman, L., Wiredu, E., Molijn, A., Quint, W., Ramakrishnan, G. & Schmidt, J. (2014). Human papillomavirus prevalence and type distribution in invasive cervical cancer in sub-Saharan Africa.*International Journal of Cancer, 134(6), 1389-1398.*

de Sanjosé, S., Diaz, M., Castellsagué, X., Clifford, G., Bruni, L., Muñoz, N. & Bosch, F.X. (2007). Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis.*Lancet Infectious Diseases, 7(7), 453-9.*

de Sanjose, S., Bosch, F.X. & Munoz, N. (1997). Social differences in sexual behaviour and cervical cancer: Social Inequalities and Cancer. *International Agency for Research on Cancer Scientific Publications,* Lyon, 309-317.

de Villiers, E.M., Fauquet C., Broker T.R., Bernard H.U. & zur Hausen H. (2004). Classification of papillomaviruses. *Virology,* 324, 17-27.

de Villiers, E.M. (2001). Human papilloma virus.*Papillomavirus Report* 12, 57

De Vuyst, H., Steyaert, S., Van Renterghem, L., Claeys, P., Muchiri, L., Sitati, S., Vansteelandt, S., Quint, W., Kleter, B., Van Marck, E. & Temmerman, M. (2003). Distribution of human papillomavirus in a family planning population in nairobi, Kenya. *Sexually Transmitted Diseases,* 30(2), 137-42. doi: 10.1097/00007435-200302000-00009.

Didelot-Rousseau , M. N., Nagot, N., Costes-Martineau, V., Vallès, X.,Ouedraogo, A., Konate, I., Weiss, H. A., Van de Perre, P., Mayaud, P., Segondy, M. & Yerelon Study Group. (2006). Human papillomavirus genotype distribution and cervical squamous intraepithelial lesions among high-risk women with and without HIV-1 infection in Burkina Faso. *British Journal of cancer,* 95(3), 355-362, doi: 10.1038/sj.bjc.6603252.

Doorbar, J. (2005). The papillomavirus life cycle. *Journal of Clinical Virology,* 32 (1), 7-15.

Elleh, N. (2001). *Abuja: The Single Most Ambitious Urban Design Project of the 20th Century*. Architektur der Welt, 5. Weimar: VDG, Verlag und Datenbank für Geisteswissenschaften. ISBN 9783897391659.

Esere, M.O. (2008). Effect of sex education programme on at-risk sexual behavior of school-going adolescents in Ilorin Nigeria. *African Health Sciences.* 8(2), 120-125.

Fadahunsi, O.O., Omoniyi-Esan, G.O., Banjo, A.A.F., Esimai, O.A., Osiagwu, D., Clement, F., Adeteye, O.V., Bejide, R.A. & Iyiola, S. (2013). Prevalence of High-Risk Oncogenic Human Papillomavirus Types in Cervical Smears of Women Attending Well Woman Clinic in Ile Ife, Nigeria. *Gynecology and Obstetrics,* 3, 6.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution,*39, 783-791.

Ferlay, J., Shin, H.R, Bray, F., Forman, D. & Mathers, C. (2010) Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer,* 127, 2893-2917.

Flores, E.R., Allen, B.L., Lee, D., Sattler, C.A. & Lambert, P.F. (1999). Cervical Cancer in women. *Virology,* 262, 344.

Flores, E.R. & Lambert, P.F. (1997). Human papilloma virus *Journal of Virology*, 71, 7167

Gravitt, P.E., Peyton, C.L., Alessi, T.Q., Wheeler, C.M., Coutl´ee, F. & Hildesheim, A. (2000). Improved amplification of genital human papillomaviruses. *Journal of Clinical Microbiology,*38, 357–361.

Grce, M., Husnjak, K., Skerlev, M., Lipozencic, J. & Pavelic, K. (2000). Detection and typing of human papillomaviruses by means of polymerase chain reaction and fragment length polymorphism in male genital lesions. *Anticancer Research,* 20, 2097– 2102.

Gheit, T. & Tommasino, M. (2011). Detection of High-Risk Mucosal Human Papillomavirus DNA in Human Specimens by a Novel and Sensitive Multiplex PCR Method Combined with DNA Microarray Methods. *Molecular Biology,* 665, 195–212.

Gillison, M. L. & Shah, K. V. (2001). Human Papilloma Virus associated head and neck squamous cell carcinoma: mounting evidence for an etiologic role for human papillomavirus in a subset of head and neck cancers**.***Current Opinion in Oncology,*13(3), 183-188.

Gomez, D.T & Santos, J. L (2007). Human papillomavirus infection and cervical cancer: pathogenesis and epidemiology. *Communicating current research and educational topics and trends in Applied Microbiology,* 117(1), 22-27.

Goodman, M.T., McDuffie, K., Hernandez, B., Wilkens, L.R., Bertram, C.C. & Killen, J. (2001). Association of methylenetetrahydrofolate reductase polymorphism C677Tand dietary folate with the risk of cervical dysplasia. *Cancer Epidemiology,* 10, 1275-1280.

Guerra- Tapia, A., Gonzalez- Guerra, E. & Rodriguez- Cerdeira, C. (2009). Common clinical manifestations of Human Papilloma Virus (HPV) Infection. *The Open Dermatology Journal,* 3, 103- 110.

Hamid, N.A., Brown, C. & Gaston, K. (2009). The regulation of cell proliferation by the papillomavirus early proteins. *Cellular and Molecular Life Sciences,*66, 1700-1717.

Harro, C. D., Pang Y. S., Roden R.B.S., Hildesheim A., Wang Z., Reynolds M.J., Mast T.C., Robinson R., Murphy B.R., Karron R.A., Dillner J., Schiller J.T. & Lowy D.R. **(**2001). Safety and immunogenicity trial in adult volunteers of a human papillomavirus 16 L1 virus-like particle vaccine.*Journal of National Cancer Institute,* 93**,** 284-292.

Hart, K.W., Williams, O.M., Thelwell, N., Fiander, A.N., Brown, T., Borysiewicz, L.K. & Gelder,C.M. (2001). Novel method for Detection, Typing, and Quantification of HumanPapillomaviruses in Clinical Samples. *Journal of Clinical Microbiology,* 39 (9), 3204- 3212.

Herrero ,R., Hildesheim, A., Bratti, C., Sherman, M. E., Hutchinson, M., Morales, J., Balmaceda, I., Greenberg, M. D., Alfaro, M., Burk, R. D., Wacholder, S., Plummer, M. & Schiffman, M. (2000). Population-based study of human

papillomavirus infection and cervical neoplasia in rural Costa Rica. *Journal of National Cancer Institutes*, 92(6), 464-74. doi: 10.1093/jnci/92.6.464.

Hildesheim, A., Herrero, R. & Castle, P. (2001). HPV co-factors related to the development of cervical cancer: results from a population-based study in Costa Rica. *British Journal of Cancer,* 84(9), 1219-1226.

Hildesheim, A., Schiffman, M.H., Gravitt,P.E., Glass, A.G., Greer, C.E. & Zhang, T. (1994). Persistence of type-specific human papillomavirus infection among cytologically normal women. *Journal of Infectious Diseases*, 169, 235–240.

Holowaty, P., Miller, A. B., Rohan, T., & To, T.(1999). Natural dysplasia of the uterine cervix. *Journal of National Cancer Institute,* 91, 252-258.

IARC, (1995). IARC monographs on the evaluation of carcinogenic risks to humans. Human Papillomaviruses, *International Agency for Research on Cancer,* 64.

IARC, (1999). IARC monographs on the evaluation of the carcinogenic risk to humans, Hormonal contraception and post-menopausal hormonal therapy, *International Agency for Research on Cancer*, Lyon 74.

Jensen, K.E., Schmiedel, S., Norrild, B., Frederiksen, K., Lftner, T. & Kjaer, S.K. (2013). Parity as a cofactor for high grade cervical disease among women with Persistent HPV: a 13-year follow up. *British Journal of Cancer.* 108(1), 234-239.

Joshi, M. &Deshpande, J.D. (2011). Polymerase Chain Reaction: Methods, Principles and Application. *International Journal of Biomedical Research,* 1 (5), 81-97.

Karlsen F., Kalantari M., Jenkins A., Pettersen E., Kristensen G., Holm R., Johansson B. & Hagmar B. (1996). *Journal of Clinical Microbiology*,34, 2095– 2100.

Keita, N., Clifford, G.M., Koulibaly, M., Douno, K., Kabba, I. & Haba, M. (2009). HPV infection in women with and without cervical cancer in Conakry, Guinea. *British Journal of Cancer.* 7, 101(1), 202–208.

Klaes, R., Woerner, S.M., Ridder, R., Wentzense, N., Duerst, M. & Schneider, A. (1999). Detection of high-risk cervical intraepithelial neoplasia and cervical cancer by amplification of transcripts derived from integrated papillomavirus oncogenes. *Cancer Research,* 59, 6132–6136.

Kleter, B., van Doorn, L. J., Schrauwen, L., Molijn, A., Sastrowijoto, S., TerSchegget, J., Lindeman, J., Ter Harmsel, B., Burger, M., & Quint, W. (1999). Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *Journal Clinical Microbiology,* 37**,** 2508-2517.

Kolawole, O., Ogah, J., Alabi, O., Suleiman, M., Amuda, O. & Kolawole, F. (2015).Utilization of Human Papillomavirus DNA Detection for Cervical Cancer Screeningin

Women Presenting with Abnormal Cytology in Lokoja, Nigeria. *Jundishapur Journal of Microbiology,* 8(10).

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution,* 35, 1547-1549.

Lamarcq, L., Deeds, J., Ginzinger, D., Perry, J., Padmanabha, S. & Smith- McCune, K. (2002). Measurements of human papillomavirus transcripts by real time quantitative reverse transcription-polymerase chain reaction in samples collected for cervical cancer screening. *Journal of Molecular Diagnostics,* 4(2), 97–102.

La Ruche, G., You, B., Mensah-Ado, I., Bergeron, C., Montcho, C. & Ramon, R. (1998). Human papillomavirus and human immunodeficiency virus infections: relation with cervicaldysplasia- neoplasia in African women. *International Journal of Cancer,* 76 (4), 480-486.

Lazcano-Ponce, E., Herrero, R., Munoz, N., Cruz, A., Shah, K.V., Alonso, P., Hernandez, P., Salmeron, J. & Hernandez, M. (2001) Epidemiology of HPV infection among Mexican women with normal cervical cytology. *International Journal of Cancer* 91, 412– 420

Lewis, M.J. (2004). Situation analysis of cervical cancer in Latin America and the Caribbean. *Pan American Health Organization* (OPS/ OMS), 1- 33.

Lie, A.K., Risberg, B., Delabie, J., Begum, S., Rimalla, R. & Hagen, B. (2004). DNA versus RNA based methods for HPV testing in screening evaluation of Hybrid Capture II and pre-tectHPV proofer in Norway. *In: Proceedings of the 21st International Papillomavirus Conference* p. 55.

Lowy, D.R.& Schiller, J.T. (2012). Reducing HPV-associated cancer globally. *Cancer Prevention Research,* (Philadelphia) 5(1), 18-23.

Luft, F., Klaes, R., Nees, M., Durst, M., Heilmann, V. & Melsheimer, P. (2001). Detection of integrated papillomavirus sequences by ligation-mediated PCR (DIPS-PCR) and molecular characterization in cervical cancer cells. *International Journal of Cancer,* 92, 9–17.

Magnani, R. (1997). IMPACT Food Security and Nutrition Monitoring Project, *Sampling guide*. Arlington, Va.

Magnusson, P. K. E., Lichtenstein, P. & Gyllenstein, U.B. (2000). Heritability of cervical tumours.*International Journal of Cancer,* 88**,** 698-701.

Manga M.M., Fowotade, A. Abdullahi, Y. M., El-nafaty, A. U., Adamu, D.B., Pindiga, H.U., Bakare, R.A. & Osoba, A.O. (2015). Epidemiological patterns of cervical human papillomavirus infection among women presenting for cervical cancer screening in North-Eastern Nigeria. *Infectious Agents and Cancer*, 10: 39

Malloy, C., Sherris, J. & Herdam, C., (2000). HPV DNA Testing: Technical Issues for Cervical Cancer Prevention. *Alliance for Cervical Cancer Prevention,* 18 (1), 1-8.

Ma´rquez, N., Paredes-Tello, M., Pe´rez-Terro´n, H., Santos-Lo´pez, G., Reyes-Leyva, J. & Vallejo-Ruiz V. (2009). Prevalence of human papillomavirus genotypes in women from a rural region of Puebla, Mexico.*International Journal of Infectious Diseases,* 13, 690-695.

Mbamara, S.U., Ukah, C.O., Ikpeze, O., Jen, O. & Onyiaora, V. (2011).Correlation between visual inspection of the cervix and Pap smear test for cervical cancer screening. *Journal of Cancer Research and Experimental Oncology*, 1, 8–13.

Mejilla, A., Li, E. & Sadowski, C.A. (2017). Human papilloma virus (HPV) vaccination: questions and answers. *Canadian Pharmascist Journal*, 150(5), 306–315.

Mirzaie-Kashani E., Bouzari M., Talebi A. & Arbabzadeh-Zavareh F. (2014). Detection of Human Papillomavirus in Chronic Cervicitis, Cervical Adenocarcinoma, Intraepithelial Neoplasia and Squamus Cell Carcinoma. *Jundishapur Journal of Microbiology*, 7(5), e9930. DOI: 10.5812/jjm.9930.

Molijn, A., Kleter, B., Quint, W. & van Doorn, L. (2005). Molecular diagnosis of human papillomavirus (HPV) infections. *Journal of Clinical Virology*, 32, 43-51.

Moreno, V., Bosch, X., Munoz, N., Meijer, J.L.M., Vshah, K., Walboomer, M.M., Herrero, R.& Franceschi, S. (2002). Effect of oral contraceptives on risk of cervicalcancer in women with human papillomavirus infection: the IARC multicentric case-control study. *The Lancet*, 359 (9312), 1080-108.

Moscicki, A.B., Hills, N., Shiboski, S., Powell, K., Jay, N., Hanson, E., Miller, S., Clayton, L., Farhat, S., Broering, T., Darragh, T. & Palefsky, J. (2001). Risks for incident human papillomavirus infection and low- grade squamous intraepithelial lesion development in young females. *Journal of American Medical Association*, 285(23), 2995- 3002. PMID: 11410098

Munoz, N., Mendez, F., Posso, H., Molano, M., Van den Brule, A.J. & Rondero, M. (2004).Incidence, duration and determinants of cervical HPV infection in a cohort of Columbian women with normal cytological results. *Journal of Infectious Diseases,* 190(12), 2077-87.

Munoz, N., Franceschi, S., Bosetti, C., Moreno, V., Herrero, R. & Smith, J.S. (2002). Role of parity and human papillomavirus in cervical cancer: the IARC multicentric case control study. *Lancet*, 359 (9312), 1093-1101.

Murray, S. (2007). Life of poverty in Abuja's wealth.*BBC News*. Retrieved June 25, 2008.
NPC: Nigeria Demographic and Health Survey. Book Nigeria Demographic and Health Survey. 2008, City.

Nascimento, S.B., Vidal, F.C.B., da Silva, M. A., Batista, J., Barbosa, M., Walbert, E. M. F.,Bezerra, G., Viana, G., Branco, R., & Brito, L. (2018). Prevalence of human papillomavirus infection among women from quilombo communities in Northeastern Brazil *BioMedCentral Womens Health.* 18, 1-2.

National Cancer Institute (2012). Pap and HPV testing. 1-2.

Nejo, Y.T., Olaleye, D.O. & Odaibo, G.N. (2019). Molecular characterisation of genital human papillomavirus among women in Southwestern, Nigeria. *PLoS ONE* 14(11): e0224748.https://doi.org/10.1371/journal.pone.0224748.

Nnodu, O., Erinosho, L., Jamda, M., Olaniyi, O. & Adelaiye, R., (2010). Knowledge and Attitude towards Cervical Cancer and Human Papillomavirus: A Nigerian Pilot Study. *African Journal of Reproductive Health,*14(14), 95-108.

Nobbenhuis, M. A., Meijer, C.J., van den Brule, A.J., Rozendaal, L., Voorhoost, F.J., Risse, E.K., Verheijen, R.H. & Helmerhorst, T.J. (2001). Addition of high-risk HPV testing improves the current guidelines on follow-up after treatment for cervical intraepithelial neoplasia. *British Journal of Cancer,* 84**,** 796-801.

Nyengidiki, T.K., Durugbo, I. & Bassey, G. (2016). Risk factors and distribution of oncogenic strains of human papilloma virus in women presenting for cervical cancer screening in Port Harcourt, Nigeria. *The Pan African Medical Journal,* 23, 85.

Okamoto, A., Woodworth, C.D, Yen, K., Chung, J., Isonishi, S., Nikaido, T., Kiyokawa, T., Seo, H., Kitahara, Y., Ochiai, K., & Tanaka, T. (1999). Combination therapy with podophyllin and vidarabine for human papillomavirus positive cervical intraepithelial neoplasia.*Oncology,* 6**,** 269-276.

Okolo, C., Franceshchi, S., Adewole, I., Thomas, J.O., Follen, M., Snijders, P.J.F., Meijer C. J.L.M. & Clifford, G.M. (2010). Human papilloma virus in women with and without cervical cancer in Ibadan, Nigeria. *Infectious Agent and Cancer,* 5, 24.

Okwuraiwe, A.P., Ohihoin, G., Audu, R.A., Achanya, D.A., Musa, Z.A., Ezechi, O.C. & Ujah, I.A.O. (2015). Characterisation of Human Papillomavirus Genotypes: Preliminary report of the Nigerian Cervical Pre-Cancer Surveillance Study.*NIMR6th Annual Scientific Conference,* Nigerian Institute of Medical Research (NIMR) Yaba, Lagos, Nigeria.

Ostor, A.G. (1993). Natural history of cervical intraepithelial neoplasia: a critical review. *International Journal of Gynecological Pathology,* 12(2), 186-192.

Park, T.C., Kim, C.J., Koh, Y.M., Lee, K.H., Yoon, J.H. & Kim, J.H. (2004). Human papillomavirus genotyping by the DNA chip in the cervical neoplasia DNA. *Cellular Biology,*23, 119–125.

Partridge, J.M.& Koutsky, L.A. (2006). Genital human papillomavirus infection in men. *Lancet Infectious Diseases.* 6(1), 21–31.

Poppe, W.A., Ide, P.S., Drijkoningen, M.P., Lauweryns, J.M. & Van Assche, F.A. (1995). Tobacco smoking impairs the local immunosurveillance in the uterine cervix: An immunohistochemical study. *Gynecologic Obstetric Investigation,* 39 (1), 34- 38.

Prokopczyk, B., Cox, J.E., Hoffmann, D. & Waggoner, S.E. (1997). Identification of tobacco-specific carcinogen in the cervical mucus of smokers and non-smokers. *Journal of National Cancer Institute,* 89 (12), 868- 873.

Public Health Service, (2001). Surgeon General's Advisory Committee on Women and Smoking. Available online: *http://www.cdc.gov/tobacco.*

Quint, W. G. V., Scholte, G., Van Doorn, L.J., Kleeter, B., Smits, P.H.M. & Lindeman J. (2001). Comparative analysis of human papillomavirus infections in cervical scrapes and biopsy specimens by general SPF_{10} PCR and HPV genotyping. *Journal of Pathology,* 194**,** 51-58.

Ramas V, Mirazo S, Bonilla S, Ruchansky D, Arbiza J. (2018). Analysis of human papillomavirus 16 E6, E7 genes and Long Control Region in cervical samples from Uruguayan women. *Gene,* 654:103-9.

Robert M.K., David A. C. & Russell E. G. (2014). Big data and large sample size: A cautionary note on the potential for bias. *Clinical and Translational Science.* 7(4): 342- 346.

Roden, R. B., Lowy, D.R. & Schiller J.T. (1997). Papillomavirus is resistant to dessication. *Journal of Infectious Diseases,* 176**,** 1076-1079.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution,* 4, 406-425.

Salvatore, V., Franceschi, S., Clifford, G.M., Touzé, A., Hsu, C.C., de Sanjosé, S. , Anh, P.T., Hieu, N.T., Thomas, J.O., Boursaghin, L., Snijders, P.J.F., Meijer, J. L.M., Muñoz, N., Herrero, R. &Coursaget, P. (2010). Seroprevalence of Antibodies against Human Papillomavirus (HPV) Types 16 and 18 in Four Continents: the International Agency for Research on Cancer HPV Prevalence Surveys. *Cancer Epidemiology Biomarkers,* 10, 1055-9965.

Sarma, U., Mahanta, J., Borkakoty, B.J., Talukdar, K.L., Gogoi, R. & Yadav, K. (2013). Demographic Characteristic of HPV Infection in Women - A Hospital Based Study from Guwahati, India. *National Journal of Medical Research.* 3(1), 1–4.

Satterwhite, C.L., Torrone, E.& Meites, E. (2013). Sexually transmitted infections among US women and men: Prevalence and incidence estimates. *Sexually Transmitted Diseases,* 40(3), 187-193.

Schnatz, P.F., Markelova, N.V, Holmes, D, Mandavilli, S.R. & O'sullivan, D.M. (2008). The prevalence of cervical HPV and cytological abnormalities in association with reproductive factors of rural Nigerian women. *Journal of Women Health,* 17, 279-85.

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

Schiffman, M., Herrero, R., Hildesheim, A., Sherman, M.E., Bratti, M., Wacholder, S., Alfaro, M., Hutcinson, M., Morales, J., Greenberg, M.D. & Lorincz, A.T. **(**2000). HPV DNA testing in cervical cancer screening. Results from women in a high-risk province of Costa Rica. *Journal of American Medical Association* 283**,** 87-93.

Schiffman, M.H., Bauer, H.M., Hoover, R.N., Glass, A.G., Cadell, D.M. & Rush, B.B. (1993). Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia. *Journal of National Cancer Institute,* 85 (12), 958- 964.

Schiller, J.T., Castellsague, X., Villa, L.L. & Hildesheim A. (2008). An update of prophylactic Human Papillomavirus L1 Virus- Like particle vaccine clinical trial results. *Vaccine,* 26(10), 53- 61.

Shikova E., Todorova I., Ganchev G. & Kouseva-Dragneva V. (2009). Detection and Typing of Human Papillomaviruses by PCR. *Biotechnology & Biotechnological Equipment,* 23 (1), 877-880.

Smith, J.S, Melendy, A., Rana, R.K. & Pimenta, J.M. (2008). Age-specific prevalence of infection with human papillomavirus in females: a global review. *Journal of Adolescent Health,* 43, 5-25.

Snoeck, R., Noel, J.C., Muller, C., De Clercq, E. & Bossens, M. (2000). Cidofovir, a new approach for the treatment of cervix intraepithelial neoplasia grade III (CIN III). *Journal of Medical Virology,* 60**,** 205-209.

Stanley, M. (2010). Pathology and epidemiology of HPV infection in females. *Gynecological Oncology,*117(2), 5-10.

SOGC (Society of Obstetrician and Gynaecologist of Canada) (2007).Human Papilloma Virus prevention. *Hpvinfo.ca*

Sun, X.W., Kuhn, L., Ellerbrock, T.V., Chiasson, M.A., Bush, T.J. & Wright T.C. (1997). Human Papillomavirus infection in women infected with the Human Immunodeficiency virus. *TheNew England Journal of Medicine,* 337(19): 1343-1349.

Syrjänen, S. M., & Syrjänen, K. J. (1999). New concepts on the role of human papillomavirus in cell cycle regulation. *Annals of Medicine,* 31**,** 175-187.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101, 11030-11035.

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

Thomas, D.B., Qin, Q., Kuypers, J., Kiviat, N., Ashley, R.L. & Koetsawang, A. (2001). Human papillomaviruses and cervical cancer in Bangkok II: Risk factors for in situ and invasive squamous cell cervical carcinomas. *American Journal of Epidemiology,* 153 (8), 732-739.

Traore, I.M., Zohoncon, T.M., Dembele, A., Djigma, F.W., Obiri-Yeboah, D., Traore, G., Bambara, M., Ouedraogo, C., Traore, Y. & Simpore, J. (2016). Molecular Characterization of High-Risk Human Papillomavirus in Women in Bobo-Dioulasso, Burkina Faso. *Biomedical Research International.*7092583.

Vaccarella, S., Franceschi, S., Clifford, G. M., Touzé, A., Hsu, C. C., de Sanjosé, S., Anh, P. T., Hieu, N. T., Matos, E., Shin, H.R., Sukvirach, S., Thomas, J. O., Boursaghin, L., Gaitan, J., Snijders, P. J. F.,Meijer, C. J. L. M., Muñoz, N., Herrero, R. & Coursaget, P. (2010). Seroprevalence of Antibodies against Human Papillomavirus (HPV) Types 16 and 18 in Four Continents: The International Agency for Research on Cancer HPV Prevalence Surveys. *Cancer Epidemiology, Biomarkers and Prevention.* 19, 2379-2388.

van den Brule, A. J., Snijders, P. J., Meijer, C. J. & Walboomers, J. M. (1993). Papillomavirus *Reports*, 4, 95-99.

Vem, T.S., Adisa, J. O., Oyero, S.K., Egbujo, E.C., Golit, W.N. & Golwa, P. (2012) Prevalence of Human Papilloma Virus Among Women Infected with Human Immuno- DeficiencyVirus. *International Journal of Current Research* 4(2), 097-099.

Veress, G., Szarka, K., Dong, P., Gergely, L., & Pfister, H.(1999). Functional significance of sequence variation in the E2 gene and the long control region of human papillomavirus type 16. *Journal of General Virology,* 80**,** 1053-1043.

Volpers, C., & Streek R. E. (1991). Genome organization and nucleotide sequence of human papillomavirus type 39. *Virology*, 181**,** 419-423.

Walboomers, J. M., Jacobs, M. V., Manos, M. M., Bosch, F. X., Kummer, J. A. & Shah, K.V. (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *Journal of Pathology,* 189 (1), 12–19.

Winer, R.L., Lee, S., Hughes, J.P., Adam, D.E., Kiviat, N.B. & Koutsky, L.A. (2003). Genital Human Papillomavirus infection: Incidence and risk factors in a cohort of female University Students. *American Journal of Epidemiology;* 157(3), 218- 226.

Winer, R.L. & Koutsky, L.A. (2004). The epidemiology of human papillomavirus infections. In:Rohan Dordrecht T, Shah K., editors. *Cervical cancer: from etiology to prevention.* The Netherlands: Kluwer Academic Publishers. pp. 143–87.

Winer, R.L., Hughes, J.P., Feng, Q., O'Reilly, S., Kiviat, N.B., Holmes, K.K. & Koutsky L. A. (2006). Condom use and the risk of genital human papillomavirus infection in young women. *The New England Journal of Medicine,* 354(25), 2645- 2654.

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

World Health Organization (WHO) – Globocan (2012). Cancer incidence and mortality worldwide: IARC Cancer Base no. 10 [internet] Lyon, France: *International Agency for Research on Cancer;* 2015.

WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre): (2014). Human papillomavirus and related diseases report in world. Available at *http://www.hpvcentre.net/statistics/reports/XWX.pdf.*

WHO (2005). Human Papillomavirus Vaccines; A New Tool for Cervical Cancer Prevention.

Xi, L.F., Kiviat, N.B., Hildesheim, A., Galloway, D.A., Wheeler, C.M., Ho, J., Koutsky, L. A. (2006). Human Papillomavirus Type 16 and 18 Variants: Race-Related Distribution and Persistence. *JNCI: Journal of the National Cancer Institute*, 98 (15), 1045–1052.

Yang, Z. &Rannala, B. (2012). Molecular phylogenetics: principles and practice. *Nature Reviews Genetics* 13, 303–314.

Yoshinouchi, M., Hongo A., Nakamura K., Kodama J., Itoh S., Sakai H., & Kudo T.(1999). Analysis by multiplex PCR of the physical status of human papillomavirus type 16 DNA in cervical cancers. *Journal of Clinical Microbiology,* 37**,** 3514-3517.

Zhai, L. & Tumban, E. (2016). Gardasil-9: a global survey of projected efficacy. *Antiviral Research* 130:101–109.

Zhang, J., Zhang, S., Wang, M., Ding, X., Wen, Q. & Chen, Z. (2018). Genetic variability in E5, E6, E7 and L1 genes of human papillomavirus type 31. *Molecular Medicine Reports*17, 5498-507.

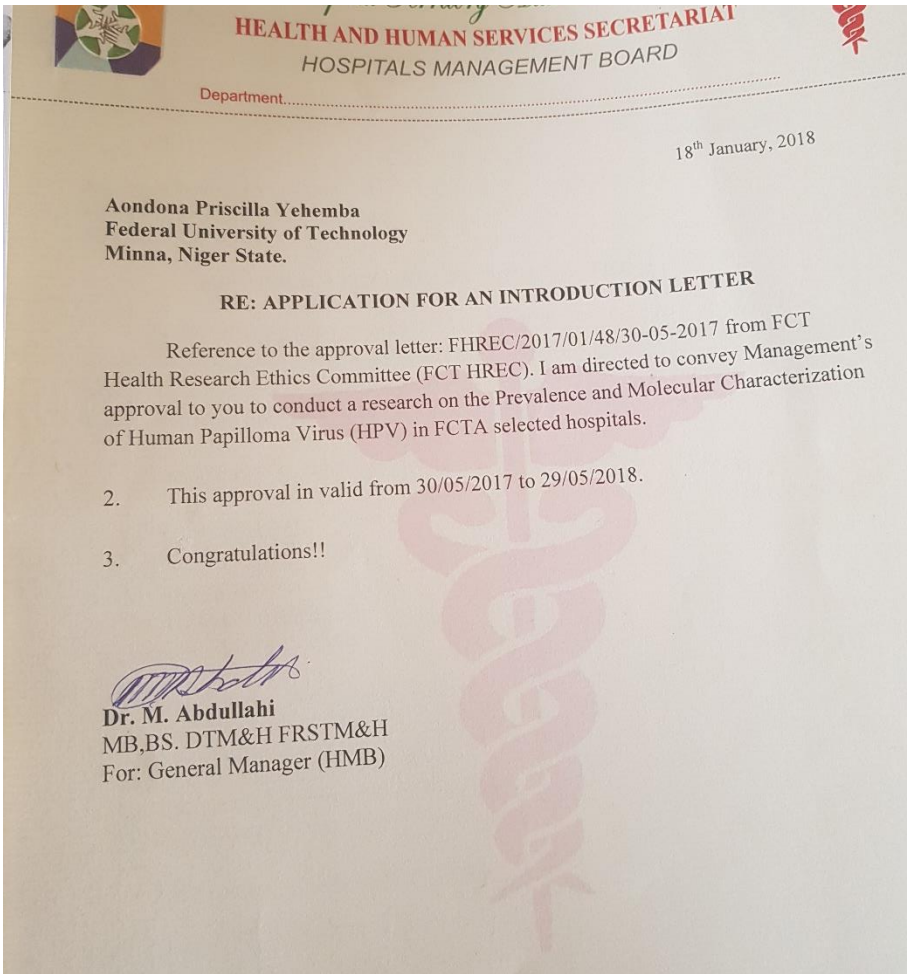
Zerbini, M., Venturoli, S., Cricca, M., Gallinella, G., P., De Simone, P., Costa, S., Santini, D., & Musiani, M. (2001). Distribution and viral load of type specific HPVs in different cervical lesions as detected by PCR-ELISA. *Journal of Clinical Pathology*.54**:**377-380.

zur Hausen H. (1996). Papillomavirus infections - a major cause of human cancers.*Biochimica et Biophysica Acta*,1288**,** 55-78.

zur Hausen H. (2009). Papillomaviruses in the causation of human cancers: a brief historical account. *Virology*, 384, 260-265.

APPENDIX A

Ethical clearance



HEALTH AND HUMAN SERVICES SECRETARIAT
HOSPITALS MANAGEMENT BOARD
Department

18th January, 2018

Aondona Priscilla Yehemba
Federal University of Technology
Minna, Niger State.

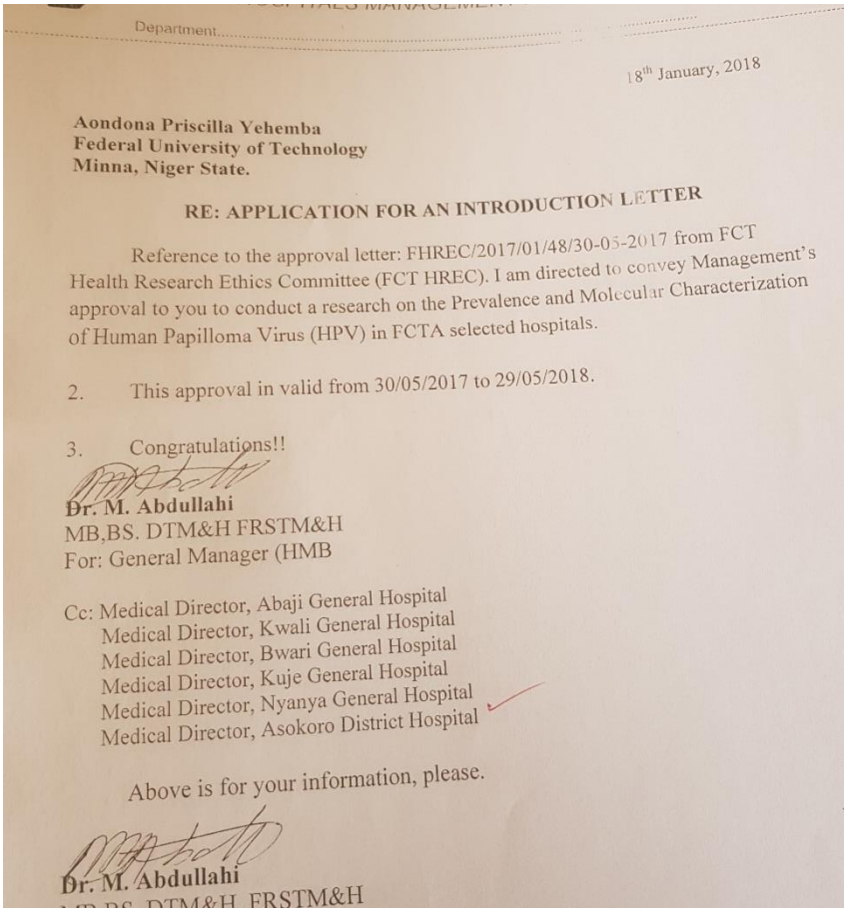
RE: APPLICATION FOR AN INTRODUCTION LETTER

Reference to the approval letter: FHREC/2017/01/48/30-05-2017 from FCT Health Research Ethics Committee (FCT HREC). I am directed to convey Management's approval to you to conduct a research on the Prevalence and Molecular Characterization of Human Papilloma Virus (HPV) in FCTA selected hospitals.

2. This approval in valid from 30/05/2017 to 29/05/2018.

3. Congratulations!!

Dr. M. Abdullahi
MB,BS. DTM&H FRSTM&H
For: General Manager (HMB)



Department

18th January, 2018

Aondona Priscilla Yehemba
Federal University of Technology
Minna, Niger State.

RE: APPLICATION FOR AN INTRODUCTION LETTER

Reference to the approval letter: FHREC/2017/01/48/30-05-2017 from FCT Health Research Ethics Committee (FCT HREC). I am directed to convey Management's approval to you to conduct a research on the Prevalence and Molecular Characterization of Human Papilloma Virus (HPV) in FCTA selected hospitals.

2. This approval in valid from 30/05/2017 to 29/05/2018.

3. Congratulations!!

Dr. M. Abdullahi
MB,BS. DTM&H FRSTM&H
For: General Manager (HMB

Cc: Medical Director, Abaji General Hospital
Medical Director, Kwali General Hospital
Medical Director, Bwari General Hospital
Medical Director, Kuje General Hospital
Medical Director, Nyanya General Hospital
Medical Director, Asokoro District Hospital

Above is for your information, please.

Dr. M. Abdullahi
MB,BS. DTM&H FRSTM&H

APPENDIX B

INFORMED CONSENT FORM

I **Aondona Priscilla Yahemba**, a PhD student of the department of Microbiology, Federal University of Technology Minna, Niger State is interested in the health and well-being of women as dangers of health issues posed on a woman will affect the entire productivity of the nation at large. To this effect I am carrying out a research on the molecular characterisation of Human Papilloma Virus (HPV) within the F.C.T.

Persistent infection with HPV is a well-established cause of genital warts, recurrent respiratory papillomatosis, Cervical Intraepithelial Neoplasia (CIN), and cancers (Malloy *et al.,* 2000). In, Nigeria the number of women at risk of cervical cancer is estimated to be 50.3million, the number of cervical cancer cases annually is estimated at 14,089 and the number of death attributed to cervical cancer is 8,240 (Bruni *et al.,* 2016).

Therefore Screening, early detection and effective treatment will reduce the overall burden of this disease in our society. The study will involve asking you questions through questionnaires and collecting sample from you. The process of sample collection will involve collecting cervical smear/swabs from the cervix (neck of the womb) which will be a little discomforting. Please I will appreciate if you decide to contribute to this study, but you might decide not to do so. The result of this investigation will be kept strictly confidential and your identity will not be disclosed. If you accept to contribute to this study please kindly sign the space provided. Thank you

Signature of Client/Date …….

Signature of investigator/Date ……

QUESTIONNAIRE

Client's Status **(Please tick appropriately)** Client's Number: ……..

1. Age

(a) 15-24 yrs { } (b) 25-34 yrs { } (c) 35-44 yrs { } (d) 45-54 yrs { }

(e) 55-64 yrs { }

2. Marital Status: Married { } Single { } Divorced { } Widowed { }

3. Occupation: Civil servant{ } Student { } Housewife { }

Business woman { } Farming { }

4. Educational Status: Formal { } Non-Formal { }

5. Number of children/ Parity: 0-2 { } 3-5 { } 6 and above { }

6. Use of Oral Contraceptives: Yes { } No { }

7.Number of sexual partners: Single { } Multiple { }

8. History of Sexually Transmitted infection (STI): Yes { } No { }

9. Age at first sexual intercourse: …….

10. Do you Smoke? Yes { } No { }

11. HIV status: Positive { } Negative { }

12. Complication History: Miscarriage { } Stillbirth { } Both { }

None { }

13. Do you know what HPV is? Yes { } No { }

14. Do you know what Cervical Cancer is? Yes { } No { }

15. Have you had a Cervical Screening Test Done before? Yes { } No { }

16. If yes how often? Once { } Twice { }Thrice or more { }

INFORMED CONSENT FORM

Introduction: I **Aondona Priscilla Yahemba**, a PhD student of the department of Microbiology, Federal University of Technology Minna, Niger State is interested in the health and well-being of women as dangers of health issues posed on a woman will affect the entire productivity of the nation at large. To this effect I am carrying out a research on the molecular characterisation of Human Papilloma Virus (HPV) within the F.C.T.

Purpose: Persistent infection with HPV is a well-established cause of genital warts, recurrent respiratory papillomatosis, Cervical Intraepithelial Neoplasia (CIN), and cancers (Malloy *et al.,* 2000). In, Nigeria the number of women at risk of cervical cancer is estimated to be 50.3million, the number of cervical cancer cases annually is estimated at 14,089 and the number of death attributed to cervical cancer is 8,240 (Bruni*et al.,* 2016). Therefore Screening, early detection and effective treatment will reduce the overall burden of this disease in our society.

Procedure of the research and what shall be required of each participant: The study will involve asking you questions through questionnaires and collecting sample from the cervix.

Expected duration of participant involvement: Participation will take about 15 minutes.

Risk: The process of sample collection will involve collecting cervical smear/swabs from the cervix (neck of the womb) which will be a little discomforting.

Benefits: Results may not be available immediately but if you need to know the outcome of your screening, adequate provisions will be made to get in contact with the participant.

Cost to the participants, if any, of joining the research: It will not cost the participant anything.

Confidentiality: The result of this investigation will be kept strictly confidential and your identity will not be disclosed.

Voluntariness: Please I will appreciate if you decide to contribute to this study, but you might decide not to do so. If you accept to contribute to this study please kindly sign the space provided below. Thank you

Signature of Client/Date …….

Signature of investigator/Date ……

Use_Contraceptives * HPV Cross tabulation

Raw Data

			HPV		
			Positive	Negative	Total
Use_Contraceptives	Yes	Count	3	43	46
		Expected Count	5.1	40.9	46.0
		% within Use_Contraceptives	6.5%	93.5%	100.0%
	No	Count	52	400	452
		Expected Count	49.9	402.1	452.0
		% within Use_Contraceptives	11.5%	88.5%	100.0%
Total		Count	55	443	498
		Expected Count	55.0	443.0	498.0
		% within Use_Contraceptives	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.055[a]	1	.304		
Continuity Correction[b]	.609	1	.435		
Likelihood Ratio	1.200	1	.273		
Fisher's Exact Test				.457	.224
Linear-by-Linear Association	1.053	1	.305		

N of Valid Cases	498				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.08.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Use_Contraceptives (Yes / No)	.537	.161	1.792
For cohort HPV = Positive	.567	.184	1.744
For cohort HPV = Negative	1.056	.972	1.148
N of Valid Cases	498		

History_sexually_transm * HPV_statusCross tabulation

Count

		HPV_status		Total
		Yes	No	
History_sexually_transm	Yes	26	174	200
	No	29	272	301

Total	55	446	501

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.393[a]	1	.238		
Continuity Correction[b]	1.070	1	.301		
Likelihood Ratio	1.373	1	.241		
Fisher's Exact Test				.246	.151
Linear-by-Linear Association	1.390	1	.238		
N of Valid Cases	501				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 21.96.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for History_sexually_transm (Yes / No)	1.402	.799	2.460

For cohort HPV_status = Yes	1.349	.820	2.221
For cohort HPV_status = No	.963	.902	1.027
N of Valid Cases	501		

Do_you_smoke * HPV_status Cross tabulation

			HPV_status		
			Yes-positive	No-negative	Total
Do_you_smoke	Yes	Count	1	4	5
		Expected Count	.5	4.5	5.0
		% within Do_you_smoke	20.0%	80.0%	100.0%
	No	Count	39	392	431
		Expected Count	39.5	391.5	431.0
		% within Do_you_smoke	9.0%	91.0%	100.0%
Total		Count	40	396	436
		Expected Count	40.0	396.0	436.0
		% within Do_you_smoke	9.2%	90.8%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.711[a]	1	.399		
Continuity Correction[b]	.004	1	.949		
Likelihood Ratio	.552	1	.458		
Fisher's Exact Test				.383	.383
Linear-by-Linear Association	.710	1	.400		
N of Valid Cases	436				

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is .46.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Do_you_smoke (Yes / No)	2.513	.274	23.041
For cohort HPV_status = Yes-positive	2.210	.373	13.086

For cohort HPV_status = No-negative	.880	.567	1.365
N of Valid Cases	436		

Number of Children * HPV_statusCrosstabulation

			HPV_status		Total
			Positive	Negative	
Number_Children	0-2	Count	31	251	282
		Expected Count	31.0	251.0	282.0
		% within Number_Children	11.0%	89.0%	100.0%
	3-5	Count	23	170	193
		Expected Count	21.2	171.8	193.0
		% within Number_Children	11.9%	88.1%	100.0%
	6 and above	Count	1	25	26
		Expected Count	2.9	23.1	26.0
		% within Number_Children	3.8%	96.2%	100.0%
Total		Count	55	446	501
		Expected Count	55.0	446.0	501.0

	% within Number_Children	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.527[a]	2	.466
Likelihood Ratio	1.926	2	.382
Linear-by-Linear Association	.207	1	.649
N of Valid Cases	501		

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 2.85.

Risk Estimate

	Value
Odds Ratio for Number_Children (0-2 / 3-5)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Complication_history * HPV_statusCrosstabulation

			HPV_status		Total
			1.00	2.00	
Complication_history	Miscarriage	Count	18	125	143
		Expected Count	15.7	127.3	143.0
		% within Complication_history	12.6%	87.4%	100.0%
	stillbirth	Count	2	17	19
		Expected Count	2.1	16.9	19.0
		% within Complication_history	10.5%	89.5%	100.0%
	Both	Count	5	26	31
		Expected Count	3.4	27.6	31.0
		% within Complication_history	16.1%	83.9%	100.0%
	None	Count	30	278	308

	Expected Count	33.8	274.2	308.0
	% within Complication_history	9.7%	90.3%	100.0%
Total	Count	55	446	501
	Expected Count	55.0	446.0	501.0
	% within Complication_history	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.707[a]	3	.635
Likelihood Ratio	1.615	3	.656
Linear-by-Linear Association	.788	1	.375
N of Valid Cases	501		

a. 2 cells (25.0%) have expected count less than 5. The minimum expected count is 2.09.

Risk Estimate

	Value

Odds Ratio for Complication_history (Miscarriage / stillbirth)	[a]

Do_you take_alcohol * HPV_statusCrosstabulation

			HPV_status		Total
			Postive	Negative	
Do_take_alcohol	No	Count	34	337	371
		Expected Count	40.7	330.3	371.0
		% within Do_take_alcohol	9.2%	90.8%	100.0%
	Mild	Count	12	68	80
		Expected Count	8.8	71.2	80.0
		% within Do_take_alcohol	15.0%	85.0%	100.0%
	Moderately	Count	9	41	50
		Expected Count	5.5	44.5	50.0
		% within Do_take_alcohol	18.0%	82.0%	100.0%
Total		Count	55	446	501
		Expected Count	55.0	446.0	501.0
		% within Do_take_alcohol	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	5.096[a]	2	.078
Likelihood Ratio	4.682	2	.096
Linear-by-Linear Association	4.972	1	.026
N of Valid Cases	501		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.49.

Risk Estimate

	Value
Odds Ratio for Do_take_alcohol (No / Mild)	[a]

a. Risk Estimate statistics cannot be computed. They are only computed for a 2*2 table without empty cells.

Age_at_first_intercourse * HPV_StatusCrosstabulation

			HPV_Status		Total
			Positive	Negative	
Age_at_first_intercourse	>18	Count	11	94	105
		Expected Count	11.5	93.5	105.0
		% within Age_at_first_intercourse	10.5%	89.5%	100.0%
	<18	Count	44	352	396
		Expected Count	43.5	352.5	396.0
		% within Age_at_first_intercourse	11.1%	88.9%	100.0%
Total		Count	55	446	501
		Expected Count	55.0	446.0	501.0
		% within Age_at_first_intercourse	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.034[a]	1	.853		

Continuity Correction[b]	.000	1	.992		
Likelihood Ratio	.035	1	.852		
Fisher's Exact Test				1.000	.507
Linear-by-Linear Association	.034	1	.853		
N of Valid Cases	501				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 11.53.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Age_at_first_intercourse (>18 / <18)	.936	.465	1.883
For cohort HPV_Status = Positive	.943	.505	1.761
For cohort HPV_Status = Negative	1.007	.935	1.085
N of Valid Cases	501		

HIV_status * HPV_statusCrosstabulation

	HPV_status	Total

			positive	Negative	
HIV_status	Positive	Count	15	49	64
		Expected Count	7.0	57.0	64.0
		% within HIV_status	23.4%	76.6%	100.0%
	Negative	Count	39	392	431
		Expected Count	47.0	384.0	431.0
		% within HIV_status	9.0%	91.0%	100.0%
Total		Count	54	441	495
		Expected Count	54.0	441.0	495.0
		% within HIV_status	10.9%	89.1%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	11.871[a]	1	.001		
Continuity Correction[b]	10.436	1	.001		
Likelihood Ratio	9.709	1	.002		
Fisher's Exact Test				.002	.001
Linear-by-Linear Association	11.847	1	.001		

N of Valid Cases	495				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 6.98.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for HIV_status (Positive / Negative)	3.077	1.582	5.986
For cohort HPV_status = positive	2.590	1.518	4.420
For cohort HPV_status = Negative	.842	.733	.967
N of Valid Cases	495		

Number_sex_partner * HPV_statusCrosstabulation

			HPV_status		Total
			1.00	2.00	
Number_sex_partner	single	Count	49	430	479
		Expected Count	52.6	426.4	479.0

		% within Number_sex_partner	10.2%	89.8%	100.0%
	Multiple	Count	6	16	22
		Expected Count	2.4	19.6	22.0
		% within Number_sex_partner	27.3%	72.7%	100.0%
Total		Count	55	446	501
		Expected Count	55.0	446.0	501.0
		% within Number_sex_partner	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.252[a]	1	.012		
Continuity Correction[b]	4.629	1	.031		
Likelihood Ratio	4.731	1	.030		
Fisher's Exact Test				.025	.025
Linear-by-Linear Association	6.239	1	.012		
N of Valid Cases	501				

a. 1 cells (25.0%) have expected count less than 5. The minimum expected count is 2.42.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Number_sex_partner (single / Multiple)	.304	.114	.813
For cohort HPV_status = 1.00	.375	.180	.780
For cohort HPV_status = 2.00	1.234	.954	1.597
N of Valid Cases	501		

Do_you_know_what is HPV_status Cross tabulation

			HPV_status		Total
			Positive	Negative	
Do_you_know_what	Yes	Count	28	227	255
		Expected Count	28.0	227.0	255.0
		% within Do_you_know_what	11.0%	89.0%	100.0%
	No	Count	27	219	246
		Expected Count	27.0	219.0	246.0
		% within Do_you_know_what	11.0%	89.0%	100.0%

Total	Count	55	446	501
	Expected Count	55.0	446.0	501.0
	% within Do_you_know_what	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.000[a]	1	.999		
Continuity Correction[b]	.000	1	1.000		
Likelihood Ratio	.000	1	.999		
Fisher's Exact Test				1.000	.556
Linear-by-Linear Association	.000	1	.999		
N of Valid Cases	501				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 27.01.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper

Odds Ratio for Do_you_know_what (Yes / No)	1.000	.571	1.752
For cohort HPV_status = Positive	1.000	.608	1.647
For cohort HPV_status = Negative	1.000	.940	1.063
N of Valid Cases	501		

Do_you know_what cervical cancer * HPV_statusCrosstabulation

			HPV_status		
			Positive	Negative	Total
Do_yknow_whatcervicalcancer	Yes	Count	26	174	200
		Expected Count	22.0	178.0	200.0
		% within Do_yknow_whatcervicalcancer	13.0%	87.0%	100.0%
	No	Count	29	272	301
		Expected Count	33.0	268.0	301.0
		% within Do_yknow_whatcervicalcancer	9.6%	90.4%	100.0%
Total		Count	55	446	501
		Expected Count	55.0	446.0	501.0

	% within Do_you know_what cervical cancer	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.393[a]	1	.238		
Continuity Correction[b]	1.070	1	.301		
Likelihood Ratio	1.373	1	.241		
Fisher's Exact Test				.246	.151
Linear-by-Linear Association	1.390	1	.238		
N of Valid Cases	501				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 21.96.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Do_yknow_whatcervicalcancer (Yes / No)	1.402	.799	2.460

For cohort HPV_status = Positive	1.349	.820	2.221
For cohort HPV_status = Negative	.963	.902	1.027
N of Valid Cases	501		

Have_you_had_Cancer Screening Test * HPV_statusCrosstabulation

Count

		HPV_status		Total
		Positive	Negative	
Have_you_had_CScrn Tst	Yes	6	41	47
	No	49	405	454
Total		55	446	501

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.170[a]	1	.680		
Continuity Correction[b]	.028	1	.868		
Likelihood Ratio	.163	1	.686		
Fisher's Exact Test				.627	.414

Linear-by-Linear Association	.169	1	.681		
N of Valid Cases	501				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 5.16.

b. Computed only for a 2x2 table

Risk Estimate

	Value	95% Confidence Interval	
		Lower	Upper
Odds Ratio for Have_you_had_CScrn Tst (Yes / No)	1.210	.489	2.995
For cohort HPV_status = Positive	1.183	.535	2.613
For cohort HPV_status = Negative	.978	.873	1.096
N of Valid Cases	501		

Age * HPV_statusCrosstabulation

			HPV_status		Total
			Positive	Negative	
Age	15-24	Count	1	20	21
		Expected Count	2.3	18.7	21.0
		% within Age	4.8%	95.2%	100.0%
	25-34	Count	18	186	204
		Expected Count	22.4	181.6	204.0
		% within Age	8.8%	91.2%	100.0%
	35-44	Count	24	168	192
		Expected Count	21.1	170.9	192.0
		% within Age	12.5%	87.5%	100.0%
	45-54	Count	8	65	73
		Expected Count	8.0	65.0	73.0
		% within Age	11.0%	89.0%	100.0%
	55-64	Count	4	7	11
		Expected Count	1.2	9.8	11.0

	% within Age	36.4%	63.6%	100.0%
Total	Count	55	446	501
	Expected Count	55.0	446.0	501.0
	% within Age	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	9.508[a]	4	.050
Likelihood Ratio	7.380	4	.117
Linear-by-Linear Association	4.493	1	.034
N of Valid Cases	501		

a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is 1.21.

Marital_status * HPV_statusCrosstabulation

			HPV_status		
			Positive	Negative	Total
Marital_status	Single	Count	8	37	45
		Expected Count	4.9	40.1	45.0
		% within Marital_status	17.8%	82.2%	100.0%
	Married	Count	40	396	436
		Expected Count	47.9	388.1	436.0
		% within Marital_status	9.2%	90.8%	100.0%
	Divorced/widowed	Count	7	13	20
		Expected Count	2.2	17.8	20.0
		% within Marital_status	35.0%	65.0%	100.0%
Total		Count	55	446	501
		Expected Count	55.0	446.0	501.0
		% within Marital_status	11.0%	89.0%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)

Pearson Chi-Square	15.390[a]	2	.000
Likelihood Ratio	11.416	2	.003
Linear-by-Linear Association	.487	1	.485
N of Valid Cases	501		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 2.20.

Occupation * HPV_status Cross tabulation

			HPV_status		
			Positive	negative	Total
Occupation	Civil servant	Count	28[a]	224[a]	252
		Expected Count	27.7	224.3	252.0
		% within Occupation	11.1%	88.9%	100.0%

	Student	Count	1_a	20_a	21
		Expected Count	2.3	18.7	21.0
		% within Occupation	4.8%	95.2%	100.0%
	House wife	Count	5_a	62_a	67
		Expected Count	7.4	59.6	67.0
		% within Occupation	7.5%	92.5%	100.0%
	Business	Count	21_a	132_a	153
		Expected Count	16.8	136.2	153.0
		% within Occupation	13.7%	86.3%	100.0%
	Farming	Count	0_a	8_a	8
		Expected Count	.9	7.1	8.0
		% within Occupation	0.0%	100.0%	100.0%
Total		Count	55	446	501
		Expected Count	55.0	446.0	501.0
		% within Occupation	11.0%	89.0%	100.0%

Each subscript letter denotes a subset of HPV_status categories whose column proportions do not differ significantly from each other at the .05 level.

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.850[a]	4	.427
Likelihood Ratio	4.942	4	.293
Linear-by-Linear Association	.101	1	.751
N of Valid Cases	501		

a. 2 cells (20.0%) have expected count less than 5. The minimum expected count is .88.

Education * HPV Crosstabulation

		HPV		
		Positive	Negative	Total
Education Primary	Count	5	54	59
	Expected Count	14.6	44.4	59.0
	% within Education	8.5%	91.5%	100.0%

	Secondary	Count	10	82	92
		Expected Count	22.8	69.2	92.0
		% within Education	10.9%	89.1%	100.0%
	Tertiary	Count	40	31	71
		Expected Count	17.6	53.4	71.0
		% within Education	56.3%	43.7%	100.0%
Total		Count	55	167	222
		Expected Count	55.0	167.0	222.0
		% within Education	24.8%	75.2%	100.0%

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	55.909[a]	2	.000
Likelihood Ratio	53.789	2	.000
Linear-by-Linear Association	42.357	1	.000
N of Valid Cases	222		

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 14.62.