

**SEROPREVALENCE OF HUMAN PAPILLOMA VIRUS TYPE 16IgG ANTIBODIES
AND ITS EFFECT ON HAEMATOLOGICAL PARAMETERS AMONG
PREGNANT WOMEN ATTENDING SOME HOSPITALS IN LOKOJA,**

KOGI STATE, NIGERIA

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DEDICATION

I dedicate this dissertation to God Almighty, the very essence of my being and the source of all knowledge and to my parents, Mr. and Mrs. Richard Haruna, the ultimate source of my inspiration.

DECLARATION

I declare that the work of this dissertation entitled “Seroprevalence of Human Papilloma Virus type 16 and its effect on haematological parameters among pregnant Women attending some Hospitals in Lokoja, Kogi State, Nigeria” has been carried out by me in the Department of Microbiology, Ahmadu Bello University, Zaria. The information obtained from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Helen Alewo, Haruna Date

CERTIFICATION

This dissertation entitled “Seroprevalence of Human Papilloma Virus and Its effect on Haematological Parameters among Pregnant Women Attending some Hospitals in Lokoja, Kogi State, Nigeria” by Helen AlewoHaruna meets the regulation governing the award of the degree of M.Sc Microbiology of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Human Papilloma Virus (HPV) infection is the primary cause of virtually all cervical cancers. Although most HPV infections are subclinical and are likely to resolve within 1-2 years, without treatment, the infection however persists in about 5-10% of infected women, leading to the development of pre-cancerous lesion which can progress to invasive cancer, 15- 20 years later. The study was carried out to determine the prevalence of Human Papilloma Virus type 16 and its effect on some haematological parameters among pregnant women attending two hospitals in Lokoja, Kogi State, Nigeria. The study was a hospital-based and cross-sectional study that combined the use of a structured questionnaire and analysis of blood samples obtained from 400 consenting women in Lokoja. The samples were analyzed for HPV type 16 IgG antibody and some immune cells using Enzyme-Linked Immunosorbent Assay and Conventional micro hematocrit titer method respectively. Both results of the positive and the negative samples were then compared with the results of the haematological parameters to see whether there's a pattern or not. Analysis of the samples gave a prevalence rate of 10% for Human Papilloma Virus type16 IgG antibodies, among the 40 respondents that tested positive to HPV type16 IgG antibodies, 80% had high WBC count while 64.9% of those negative had high WBC counts. 95% of them had high lymphocyte counts while 0.8% of those without the HPV IgG antibodies had high lymphocyte count. About 87.5% of those that had the infection had high monocyte counts while 41.6% of those without the HPV IgG antibodies had high monocyte counts. Approximately 82.5% of those with the HPV 16 IgG antibodies had high neutrophil count and only 0.6% of those without the antibodies had high neutrophil count, 5% of those with the HPV 16 IgG antibodies had high basophil counts while 4.7% of those without the HPV16 antibodies had low basophil counts. A change in pattern was observed with the eosinophil count where 5% of those with the antibodies had high counts and 8% of those without the HPV IgG antibodies had high counts and PCV where 2.5% of those without the HPV IgG antibodies had high counts and 8.6% of those without the infection had high counts. Socio-demographic factors associated with the presence of HPV type 16 antibodies in the study were occupation, level of education and type of marriage (polygamy) while marital status and age were not significantly correlated to presence of HPV type 16 antibodies. The risk factors observed for the infection were early sexual initiation, multiple sexual partners and high parity. The prevalence of HPV type 16 antibodies found in this study called for the institution of HPV awareness campaigns programs and the implementation of a routine cervical cancer screening in all states of the federation. It also emphasizes the need for more research work on HPV type 16 infection.

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CHAPTER ONE

1.0

INTRODUCTION

1.1 Background of the Study

Human papillomavirus (HPV) is a non-enveloped deoxyribonucleic acid (DNA) virus belonging to the family *papillomaviridae*. This family includes more than 130 genotypes, many of which infect the mucosal areas of the human upper digestive tract and the anogenital region through sexual contact, leading to increased risk of development of cancer (Frazier, 2010; Touze *et al.*, 2010). These genotypes are grouped into "high risk" and "low risk" according to the degree of risk of development of cancer after infection. Infection with the high risk serotypes of HPV can lead to cervical cancer and are associated with other mucosal anogenital, and head and neck cancers (Bosch *et al.*, 1995; Chew *et al.*, 2005). Infection with the low-risk serotypes, is known to cause benign or low-grade cervical tissue changes and genital warts (condyloma acuminata) on the cervix, vagina, vulva, and anus in women and on the penis, scrotum, and anus in men (CDC, 2009).

Human papillomavirus infection is one of the main causes of sexually transmitted diseases in the world, especially in developing countries where the prevalence of the asymptomatic form varies from 2 to 44%, depending on the population (Bosch and De-Sanjose, 2003). Evidence shows that most sexually active individuals are exposed to infection from this virus at some moment in their lives (Trottier *et al.*, 2008).

Among the risk factors for HPV infection are the following: age at first sexual intercourse, number of sexual partners, parity, smoking and other sexually transmissible infections.

Moreover, oral contraceptive use, immune and nutritional status, and genetic background of the host are also considered risk factors for HPV infection (Matsumoto *et al.*, 2003; Simen *et*

al.,2008). More than 120 different HPV types have been catalogued so far, and about 40 serotypes infect the epithelium of the anogenital tract and other mucosal area of the body. At least 15 of these oncogenic or high-risk HPV (HR-HPV) are strongly associated with progression to invasive cervical cancer (Munoz *et al.*, 2003).The most prevalent type found in all the studies was HPV 16. However, the prevalence of both HPV 16 and the other types differs considerably according to the degree of the geographic region (Cliford *et al.*, 2006). Although most HPV infection resolves spontaneously, a small fraction of infected individuals, known as chronic or persistent carriers, will retain the virus without exhibiting any clinical manifestation. It is now well established that persistent infection with HR-HPV is necessary condition, although it is not enough to provoke the development of cervical cancer and its precursor lesions (Bosch *et al.*, 1999).

1.2 Statement of Research Problem

The Human Papilloma Virus (HPV) infection is the most common sexually transmitted disease (STD) worldwide, representing a significant health problem due to its high prevalence and transmissibility (Trim *et al.*, 2012). It is estimated that 75 percent of the sexually active population has been exposed to HPV (Koutsky, 1997). Prevalence estimate varies according to the diagnostic method and the population examined, with higher rates being observed in studies using molecular biology and including young women with high- risk sexual behavior (Trottier and Franco, 2006).Virtually all cases of cervical cancer worldwide are caused by persistent infection with one or more of approximately a dozen carcinogenic genotypes of Human Papilloma Virus (Schiffman *et al.*, 1996; Walboomer *et al.*, 1999; Munoz *et al.*, 2003)

In a recent meta-analysis, global HPV prevalence in North America and Europe was estimated at 21% with sub Saharan Africa topping the list at 24% (Dahlstrom *et al.*, 2010; Ezenwa *et al.*, 2013). In Nigeria, the prevalence of HPV is high in all female groups and highest in women aged

15-23yrs (Ezenwa *et al.*, 2013; Bruni *et al.*, 2014)

Cervical cancer resulting from persistent infection with HPV is an important health problem worldwide and the second most common cancer among women and ranking first in many developing countries (Di *et al.*, 2008; Rock *et al.*, 2000). Half a million women develop cervical cancer annually and more than half die from the disease (Di *et al.*, 2008).

Cervical cancer is a preventable disease if there is proper awareness and regular screening of infections for high-risk HPV, but has continued to pose a huge management challenge to the modern day gynecologist due to vague knowledge about the virus (Munoz *et al.*, 2003). It is the leading cause of death per annum in women aged 35-45 years (Papadopoulous, 2000).

In 2008, more than 27,000 women died of cervical cancer worldwide, with nearly 85% of these deaths occurring in developing countries (Bruni *et al.*, 2014; Nnodu *et al.*, 2010). In Nigeria, the most populous country in sub-Saharan Africa, it is the second most common cancer (after breast cancer) where it has been estimated that of the 14,550 women who are diagnosed with the disease, 9659 die from it annually (WHO, 2010).

Researchers hypothesized that during pregnancy, high progesterone levels may activate transcriptional HPV infection or change the immune response. Other studies have demonstrated that increasing parity is linked with cervical cancer (Smith *et al.*, 2012).

1.3 Justification of the Study

Nigeria has a high cervical cancer incidence (ASR at 29.0 per 100,000 women/year) and has a low cervical cancer screening *in* both urban and rural areas. The low coverage of screening may be due to lack of awareness. Previous studies done in many parts of the world and especially in sub Saharan Africa have revealed low knowledge in Human Papilloma virus and its precursor

lesions in the development of cervical cancer. No such study to the best of my knowledge has been published in Kogi State among pregnant women and relating it to hematology results. A study on the seroprevalence of Human Papillomavirus, knowledge and awareness of cervical cancer and its prevention could serve as a platform for setting up prevention programs which would include educational campaigns and screening services. It is anticipated that educational campaigns would lead to increase in awareness and this would in turn lead to good health seeking behavior. In the absence of adequate knowledge and awareness, screening would still not yield results, as observed by Tebeu. (2008).The primary justification for this study was to find out the seroprevalence of Human papillomavirus type 16 igG antibodies.

Among the prevention methods of cervical cancer, HPV vaccination has been developed as a primary prevention measure and it is being administered in a number of countries including twenty (20) countries in Africa, despite poor knowledge on HPV as a cause of cervical cancer. HPV vaccine has been highly accepted as a primary prevention for cervical cancer in many countries including Nigeria.

1.4 Aim of the Study

The aim of this study was to determine the seroprevalence of Human Papilloma virus type 16 and its effects on some hematological parameters among pregnant women attending clinics in two hospitals in Lokoja, Kogi State, Nigeria.

1.5 Objectives of the Study

The objectives of this research work were to:

- 1 Determine the sero-prevalence of human papilloma virus type 16 IgG antibodies among pregnant women attending clinics in the study population using ELISA techniques.
- 2 Determine the socio-demographic and possible risk factors associated with human papilloma

virus type 16 in pregnant women using structured questionnaire.

- 3** Determine the haematological patterns due to human papilloma virus type 16 infections in women attending clinics in two hospitals in Lokoja, Kogi State.

CHAPTER TWO

2.0

LITERATURE REVIEW

2.1 Background to Study

The Human papillomavirus infection is the sexually transmitted infection most frequent in man and woman (Koutsky, 1997; Worda *et al.*, 2005). The probability of transmission of HPV through sexual intercourse varies from 5 to 100% with an average of 40%. The probability of transmission per partner (male-female) is estimated at 60% for HPV 16 and 60% for HPV that causes genital warts. Detection of HPV DNA by the molecular biological methods does not necessarily represent the manifestation of a disease (Burchell *et al.*, 2006).

According to the World Health Organization (WHO), more than 630 million men and women (1 in every 10 people) are infected with HPV in the world (Ferlay *et al.*, 2004; CDC, 2007). Clinical manifestation is present in less than 10% and the infection is often asymptomatic and can be unnoticed by the patient even though a lesion is present. It is believed, that approximately 1/3 of all women in the world have some form of clinically manifested HPV infection. Also, half of all the women of the world sexually active population are infected with this virus (Snoeck, 2006). It is believed as well that after exposure to HPV, the virus infects the entire lower genital tract epithelium (Shepherd and Bryson, 2008). The incubation period is highly variable, ranging from a few days to many years (20 to 30 years or more) (Sinal and Woods, 2005). When the infection process starts, there is a proliferation phase ranging from 3 to 6 months, when many lesions appear. After the response of B and T cells to the infection, what follows is the containment phase that also lasts for 3 to 6 months, when regression will occur to more than 80% of the lesions. The remaining 20% will have an active disease or recurrence after variable disease-free intervals (Franco and Steben, 2007). The clinical manifestations are variable and are associated with systemic and local immune response of each individual, with different

environmental factors.

Dependent on the host immune system, the course of the infection can take one of the three following forms. The most frequent is the Latent Infection, where no clinical manifestation of the infection occurs, and it is only detected by the HPV DNA detection methods. The second form is the Subclinical Infection with minimal clinical manifestation that is usually diagnosed by colposcopy, cytology and histology. The third form that is the least common is the Clinical Infection. In this form there is an active expression of the disease, manifested mainly by genital warts, precancerous lesions and invasive cancer (Chow *et al.*, 2010). Different manifestations are also dependent on different types of HPV (currently more than 200 types) and also the host immune system (Bernard, 2005). The low-risk HPV will mainly produce warts (condyloma) and the high risk HPV will mainly produce an intraepithelial lesion (Trofatter, 1997).

2.2 Natural History of HPV Infection

The natural history of HPV infection is dated back to 1972 when the association of the human papillomaviruses with skin cancer in epidermodysplasia verruciformis was proposed by Stefania Jablonska in Poland in 1978. Jablonska and Gerrard Orth at the Pasteur institute discovered HPV-5 in skin cancer (WHO 2007) in 1976. Harald Zur Hausen published the hypothesis that human papilloma virus plays an important role in the cause of cervical cancer. In 1983 and 1984 Zur Hausen and his collaborators identified HPV16 and HPV18 in cervical cancer (WHO 2004). The HeLa line contains extra DNA in its genome that originated from HPV type 18 (WHO 2007) Human papillomaviruses.

HPV is a small DNA virus with a genome of approximately 8000 base pairs (Scheurer *et al.*, 2005). HPV targets the basal cells in the stratified squamous epithelium and the metaplastic cells at the squamocolumnar junction of the cervix. Additionally, HPV may infect the glandular epithelium of the endocervix, resulting in glandular neoplasms, such as adenocarcinoma in situ

or invasive adenocarcinoma (Longworth *et al.*, 2004). The two primary oncogenes of high-risk HPV types are E6 and E7. The “E” designation indicates that these two genes are expressed early in the HPV life cycle. The products of these two genes alter host-cell metabolism to favor neoplastic development. E6 binds to and degrades the host-cell protein p53. An effect of this targeted degradation is to prevent apoptosis of the infected host epithelial cells. Telomerase is also activated, further augmenting oncogenic changes. The E7 protein has a similar effect on cell metabolism by binding to retinoblastoma protein, inhibiting its function. This leads to disruption of the cell cycle (Scheurer *et al.*, 2005). In addition, E6 and E7 proteins may cause chromosomal destabilization, and inhibit cyclin-dependent kinase inhibitors and host interferon. (Zur-Hausen, 2000).

2.3 Classification of Human Papilloma Virus

Human papillomaviruses (HPVs) are small, double-stranded DNA viruses that infect cutaneous and mucosal epithelial tissues of the anogenital tract, the hands, or the feet. Subsets of HPV types are the causative agents of cervical cancer, since 99% of tumors are positive for HPV DNA (Tommasino *et al.*, 1993). To date, over 100 different viral types have been identified, and about one-third of these infect epithelial cells in the genital tract. The viral types that infect the genital tract fall into two categories: high risk and low risk. The high-risk types are associated with the development of anogenital cancers including those of the cervix, while infections by the low-risk HPVs induce only benign genital warts. The high-risk types include HPV-16, HPV-18, HPV-31, HPV-33, and HPV-45, while the low-risk types are HPV-6 and HPV-11. HPVs that infect the genital tract are sexually transmitted, and it is estimated that about two-thirds of individuals who have sexual relations with an infected partner will themselves become infected. However, the majority of infections are subclinical (Sedman and Stelund, 1998). Infection by high-risk HPVs is not limited to the genital tract, since approximately 20% of cancers of the oropharynx contain

DNA from these HPV types (Hererro *et al.*, 2003)

2.4 Structure of Human Papillomavirus

Human papillomaviruses are non-enveloped viruses with icosahedral capsids that replicate their genomes within the nuclei of infected host cells. The double-stranded, circular DNA genomes of all HPVs are approximately 8 kb in size. In virions, the HPV DNA is found associated with cellular histones to form chromatin-like complexes (Oh *et al.*, 2001). The viral genomes carry on average eight major open reading frames (ORFs), and these are expressed from polycistronic mRNAs transcribed from a single DNA strand. In the high-risk HPV types, transcripts are initiated at two major viral promoters, one of which initiates upstream of the E6 open reading frame, encodes early viral proteins, and is expressed prior to productive replication. In HPV-16 and HPV-31 this promoter is referred to as p97, while in HPV-18 it is referred to as p105. Coincident with the induction of productive replication, the late promoter is activated, which directs expression from a series of heterogeneous start sites clustered around nucleotide 742 (p742) in HPV-31 (Hummel *et al.*, 1992). Similar promoters have been identified in HPV-16 and HPV-18 (Frattoni *et al.*, 1994, Grassman *et al.*, 1996). Several additional minor promoters have been identified that also play important roles during the viral life cycle (Oh *et al.*, 2001).

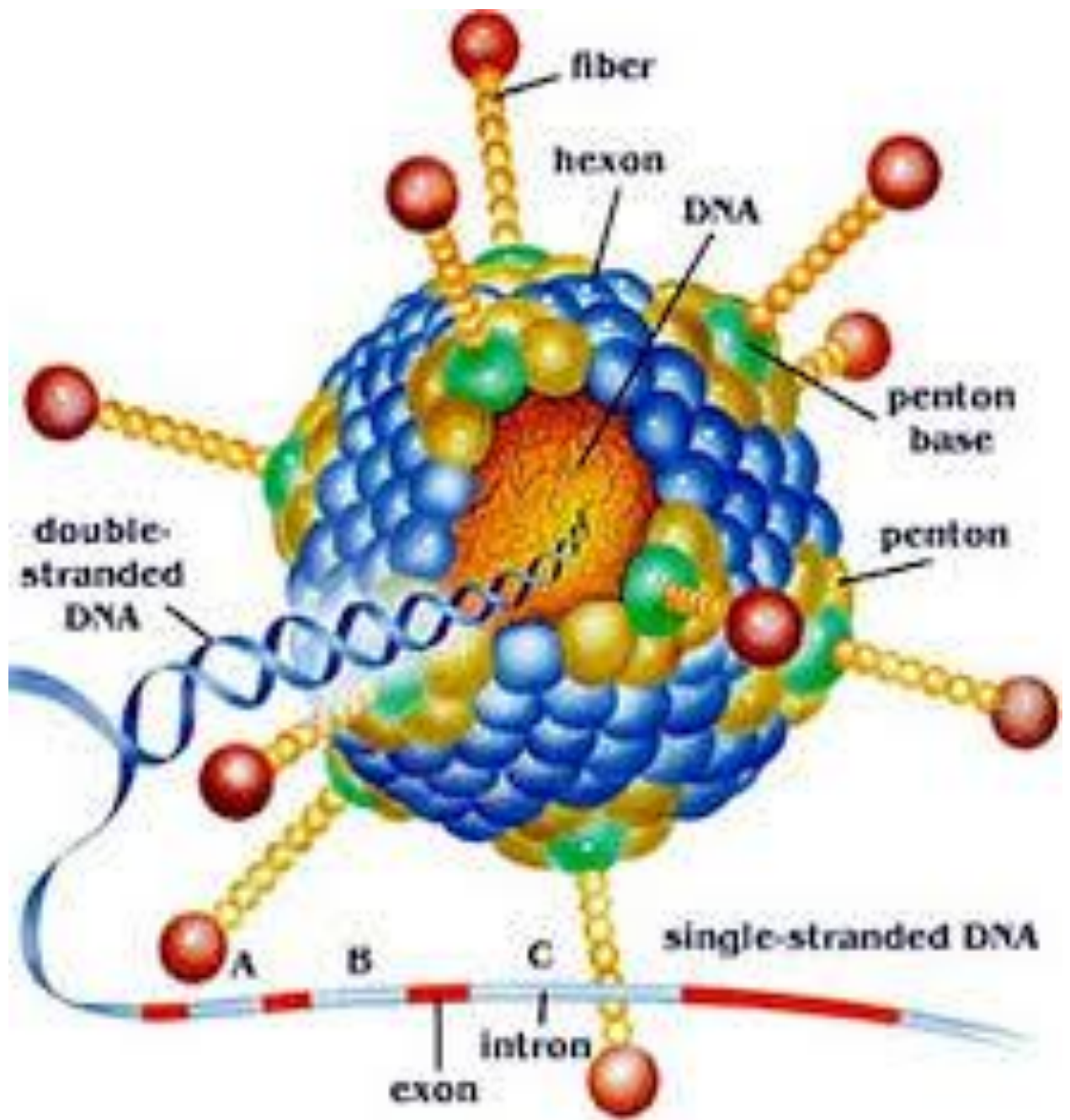


Figure 2.1 Typical Structure of Human Papillomavirus

2.5 Transmission of HPV

These viruses cause the formation of warts on the skin, mucous membranes of the oral cavity, respiratory tract, throat and genitals and also are the cause of urethral warts. The upper respiratory tract infection is possible through two mechanisms: transmission from mother to child by the infected birth canal and through sexual contacts (Szkoda *et al.*, 1995 and Bharti *et al.*, 2013) Skin injuries, micro injuries of the epidermis and the mucous membranes, and skin abrasion (Szkoda *et al.*, 1995) and (Beuchner, 2002) are additional way for the virus to enter the body. These factors facilitate the penetration of the virus into the stem cells of the basal layer. Infection causes growth stimulation and the formation of pathologic cells, additionally in the virus replication; the correlation with differentiating epidermal cells is visible. Virions are also present in superficial layers, which facilitate the transfer of infection during contact (Szkoda *et al.*, 1995; Kaplana *et al.*, 2003 and Hafkamp *et al.*, 2004)

American Centers for Disease Control and Prevention report of 2013 shows that HPV constitutes the majority of newly acquired STI's (sexually transmitted infections). Approximately 20 million new cases of STI's are reported annually, among which HPV is the most common sexually transmitted virus in the USA. Nearly 200 strains of HPV have been enumerated, majority of which are harmless, however over 40 of them can infect genital and oral mucosa of both males and females, and out of all 9 are considered to cause cancer. Each day approximately 12 000 American residents aged 15–24 are infected with genital HPV (Chow *et al.*, 2007). There are many ways of acquiring oral and oropharyngeal HPV infections such as sexual contacts and kissing between partners who are infected. Vertical transmission from mother to child during childbirth is also possible. Benign lesions of the upper aerodigestive tract may also be caused by mucosotropic human papillovirus strains (CDC, 2013).

2.6 Replication of Human Papillomavirus

The mode of viral replication is closely coupled to the differentiation status of the infected squamous epithelium (Chow and Broker, 1997). In the basal and parabasal cells of the squamous epithelium, the virus is maintained as a low copy number extra-chromosomal episome and undergoes regulated DNA replication modulated by both viral and host proteins. As cells undergo progressive differentiation, vegetative viral replication is triggered, “late” viral genes are expressed, and progeny virions are produced in a fraction of the terminally differentiated cells in papillomas or condylomas. The latent stage of papillomaviral replication provides an ideal system for the study of regulated eukaryotic DNA replication.

Different authors (Yang *et al.*, 1998; Chow *et al.*, 1994) have reported a cell-free replication system for bovine papillomavirus type 1 (BPV-1)¹ (Yang *et al.*, 1991) and human papillomavirus type 11 (HPV-11) (Chow *et al.*, 1996). Papillomaviral replication requires the viral proteins E1 and E2 (Sedman and Stelund, 1996; Chow and Broker, 1994), as well as the full complement of host replication proteins that have previously been identified in SV40 *in vitro* replication, including DNA polymerases α and δ (Hassel and Brinton, 1996). It is therefore probable that physical interactions between the host initiation enzymes and the papillomaviral initiation proteins E1 or E2 occur during initiation of viral DNA replication.

The papillomaviral E1 protein is a functional homolog of SV40 large T antigen, with origin binding activity as well as ATPase and helicase activity (Hughes and Romanus, 1993, Yang *et al.*, 1991; Seo *et al.*, 1993; Bream *et al.*, 1993; Gillete and Borowie, 1998). It associates as a trimer or a hexamer on its cognate E1-binding site in the viral origin (ori) with relatively low affinity and low sequence specificity (Sedman and Stelund 1995; Holt *et al.*, 1994; Sedman *et al.*, 1997). As a result, high concentrations of E1 can bind DNA nonspecifically and initiate ori-independent replication at low efficiency *in vitro* (Chow *et al.*, 1994; Yang *et al.*, 1999). It has been proposed that the replication competent form of BPV-1 E1 is a multimeric complex of 10–12 E1 molecules

(Lusky *et al.*, 1994). Recently, the HPV-11 E1 protein has been shown to bind to the human chaperone protein Hsp40, and in its presence, a dihexameric E1 complex forms on the ori (Liu *et al.*, 2001), mirroring the structure of SV40 T antigen on the SV40 ori (Wessel *et al.*, 1992) as well as other known *Escherichia coli* helicases (West, 1996). In addition to its role in initiation, HPV-11 E1 is also required during elongation *in vitro*, suggesting its helicase activity may be critical at the replicating forks (Liu *et al.*, 1995).

The E2 is a viral transcriptional transactivator that is essential for viral DNA replication *in vivo* (Ustav and Stelund, 1991; Chiang *et al.*, 1992). It binds as a dimer with high affinity to its conserved binding sites (E2-binding site) in the viral genome, including several sites in the viral origin of replication (Chiang *et al.*, 1992). It forms a complex with E1 and this E1·E2 complex has increased sequence specificity for binding to the E1 and E2 cognate sites in the viral origin (Fratinni and Liamins 1994). Thus one of the critical functions of E2 in viral DNA replication is to interact with and recruit E1 to the viral origin of replication by virtue of the stronger DNA binding affinity and specificity for E2 (Berg and Stelund 1997, Zou *et al.*, 1998). Therefore, the addition of E2 protein to a cell-free replication assay enhances ori-dependent and suppresses ori-independent replication.

Based on these data the following model of E2/E1 interaction during initiation of bovine papillomaviral DNA replication has been proposed (Lusky *et al.*, 1994). Once the first molecule of E1 is loaded onto the origin by E2, E2 is then released from the origin, allowing E1 to multimerize into a replication-competent form. However, more recent studies in HPV replication have suggested that the role played by E2 extends beyond the recruitment of E1; E2 is necessary for the formation of the entire pre-initiation complex, although it is dispensable during elongation (Sedman and Stelund, 1995). This interaction between E2 and E1 is extremely important for HPV ori replication, as only the E2-binding site is absolutely essential for origin-

specific viral replication, whereas the E1-binding site is dispensable for *in vivo* or *in vitro* viral DNA replication (Chow *et al.*, 1994, Chang *et al.*, 1992). In addition, certain heterologous combinations of E1 and E2 either do not support replication, or do so poorly, whereas the homologous pairs of viral proteins always support replication effectively (Chiang *et al.*; 1992; Berg and Stelund 1997), suggesting type-specific interactions between E1 and E2.

The DNA pol α /primase is the principal enzyme for the initiation of DNA synthesis and is required for both leading and lagging strand DNA replication (Wang and Collins, 1996). Pol α /primase is essential for the initiation of cell-free replication of SV40 (Donreiter *et al.*, 1993), BPV-1 (Park *et al.*, 1994) and HPV-11 (Chow *et al.*, 1994). DNA pol α /primase has also been shown to be the major host factor responsible for species-specific replication of SV40 and polyomaviral DNA *in vitro* (Park *et al.*, 1994). These findings not only indicate the critical function of DNA pol α /primase in the initiation of viral DNA replication but also reveal the important role played by this enzyme complex in the timing and control of DNA replication in eukaryotic cells. pol α /primase consists of four subunits as follows: a polymerase catalytic subunit of 180 kDa (p180), two smaller subunits of 58 and 49 kDa (p58 and p49) containing the primase activity, and a 70-kDa subunit (p70 or B-subunit) which is a cell cycle-dependent phosphoprotein (Foiani *et al.*, 1995) with no known catalytic function (Wang, 1991). The catalytic subunit p180 has been shown to interact physically with both the SV40 T antigen (Donreiter *et al.*, 1993) and with the BPV-1 E1 protein (Park *et al.*, 1994; Bonne Andrea *et al.*; 1995), and the interaction between p180 and T antigen is required for SV40 viral DNA replication *in vitro* (Donreiter *et al.*, 1993). The p70 subunit has also been shown to interact physically with SV40 T antigen *in vitro*, although the functional significance of this interaction is not understood (Collins *et al.*, 1993). DNA polymerase α activity is essential for the initiation of cell-free replication of SV40 (Seo *et al.*, 1993; Park *et al.*, 1994), and HPV-11 (Chow *et al.*, 1994). It is probable that analogous interactions between DNA pol α /primase and the host

counterparts of the viral initiators may also play a critical role in the control of chromosomal DNA replication.

2.7 Pathogenesis of Human Papillomavirus

The viral genome of the HPV consists of a single molecule of double-stranded and circular DNA, containing approximately 8000 base pairs and harboring an average of 8 open reading frames (ORFs) (Jo and Kim, 2005). In a functional point of view, the HP genome is divided into three regions. The first is a noncoding upstream regulatory region (URR) or long control region (LCR) that has regulatory function of the transcription of the E6 and E7 viral genes; The second is an early region (E), consisting of six ORFs: E1, E2, E4, E5, E6, and E7, which encodes no structural proteins involved in viral replication and oncogenesis. The third is a late (L) region that encodes the L1 and L2 structural proteins. The LCR region of the anogenital HPVs ranges in size between 800-900 pb, representing about 10% of the genome, and varies substantially in nucleotide composition between individual HPV types (Jo and Kim, 2005). Only one strand of the double-stranded DNA serves as the template for viral gene expression, coding for a number of polycistronic mRNA transcripts. (Stanley, 2008). The regulation of viral gene expression is complex and controlled by cellular and viral transcription factors. Most of these regulations occur within the LCR region, which contains cis-active element transcription regulators. These sequences are bound by a number of cellular factors as well as the viral E2 product (Zur-Hausen, 1996). A large number of cellular transcription factors have been identified and the dysfunction of some of them appears to play a significant role in papillomavirus-linked carcinogenesis. The transcription start sites of viral promoters differ depending on the virus type, but, in all types, promoter usage is keratinocyte differentiation-dependent (Smith *et al.*, 2007). The replication origin and many transcriptional regulatory elements are found in the upstream LCR region. The virus early promoter, differentiation-dependent late promoter, and two poly-adenylation signals

define three general groups of viral genes that are coordinately regulated during host cell differentiation.

The E6 and E7 genes maintain replication competence. E1, E2, E4, E5, and E8 are involved in virus DNA replication, transcriptional control, beyond other late functions and L1 and L2, responsible for the assembly of viral particles (Leuchner and Laimins, 2011). The regulation of expression of the late genes in genital HPVs is not well understood. However, it has been shown that the second, or later, promoter is initiated in a differentiation-dependent manner, and thus is activated only when cells are grown in the host's stratifying/differentiating tissue. Once activated, the later promoter directs transcription from a heterogeneous set of start sites and will serve to produce a set of transcripts that facilitate the translation of L1 and L2 proteins (Smith *et al.*, 2007). Activation of the later promoter is accompanied by acceleration of viral DNA replication and by high levels of viral protein expression. As a result, virus copy-number amplifies from 50 copies to several thousands of copies per cell. So when a late promoter is activated, the expression of genes will occur, encoding the structural proteins L1 and L2, which join to assemble the capsids and to form virions (Stanley, 2008).

2.8 Functions of Viral Proteins

2.8.1 The E1 Protein

The E1 protein represents one of the most conserved proteins among different HPV types. It has DNA-binding functions and a binding site in the origin of replication localized in the LCR region. It assembles into a hexameric complex, supported by the E2 protein, and the resultant complex has helicase activity and initiates DNA bidirectional unwinding, constituting a prerequisite for viral DNA replication (Frattini and Laimins, 1994). The carboxyl terminal domain of E1 has an ATPase/helicase activity and is necessary and sufficient for oligomerization. This domain also interacts with the E2 protein and subunit p70 of DNA polymerase α , but is not

sufficient to support replication (Amin *et al.*; 2000). A segment of approximately 160 amino acid residues upstream of the ATPase/helicase domain is the DNA-binding domain. A stretch of about 50 amino acids within the amino terminus of E1 acts as a localization regulatory region (LCR) and contains a dominant nuclear export sequence (NES) and a nuclear localization signal (NSL), which are regulated by phosphorylation (Deng *et al.*, 2004).

2.8.2 The E2 Protein

The E2 open reading frame of HPV gives rise to multiple gene products by alternative RNA splicing. The proteins derived from the E2 gene are involved in the control of viral transcription, DNA replication, and segregation of viral genomes (McPhillips *et al.*; 2006; Kadaja *et al.*; 2009). These different E2 types represent the major intragenomic regulators (Bouvard *et al.*; 1994). The E2 protein can bind to factors on mitotic chromatin and join the virus genome to host cell chromosomes during mitosis; it contributes to coordinating the HPV DNA replication with host cell chromosome duplication, allowing the viral genomes to be distributed to the daughter cell. This constitutes an important requirement for the persistence of virus DNA in undifferentiated basal cells (McPhillips *et al.*, 2006). Furthermore, the E2 protein interacts with E1 and stimulates viral DNA replication, favoring the binding of E1 to the origin of replication (Seo *et al.*, 1993; Chow *et al.*, 1994). In lesions containing HPV episomes, the E2 protein directly represses the expression of early genes as a mechanism to regulate the copy number. In addition, it has been reported that HPV E2 proteins are able to repress telomerase promoter activity mediated by the HPV E6 protein (Hamid *et al.*, 2009). Integration of the HPV genome in the host cell chromosome usually disrupts E2 expression, causing a deregulated expression of early viral genes, including E6 and E7, and this event can favor the transformation of human cells and the transition into a malignant state (Romanczuk and Howley, 1992). In addition to the full-length E2 protein, the infected cells can express an E8^{E2C} transcript, in which the small E8 domain is

fused to the C-terminal domain of E2 (E2C). The full-length E2 protein forms heterodimers with repressor forms of E2, and these E2 heterodimers serve as activators of transcription and replication during the viral cycle.

The single-chain E2 heterodimer in the HPV 18 genome initiates genome replication, but is not sufficient for long-term replication of the HPV 18 genome. This is due to the capacity of HPV18 in encoding the repressor E8/E2, which acts as a negative regulator of HPV18 genome replication (Kurg *et al.*, 2010). Moreover, it has been shown that inactivation of E2 in the HPV16 genome increases E6/E7 transcription and that mutation of E8^{E2C} in the HPV31 or HPV16 genome increases the genome copy number and the E6/E7 transcription, suggesting that the transcriptional repressing by E8^{E2C} has an important role in viral replication (Lace *et al.*, 2008). It was also noted that the E2C domain not only mediates specific DNA binding but has also an additional role in transcriptional repression by recruitment of co-repressors, such as the CHD6 protein. This suggests that repression of the E6/E7 promoter by E2 and E8^{E2C} involves multiple interactions with host cell proteins through different protein domains (Fertey *et al.*, 2010)

2.8.3 The E4 Protein

Despite being considered an early protein, E4 is exclusively located in the differentiated layers of the infected epithelium (Zur-Hausen, 1996). Although its expression occurs in highly differentiated cells that express the capsid genes and synthesize new progeny virions, and coincides with the onset of vegetative viral DNA replication, E4 is not found in virion particles. The role of this protein in the virus life cycle has not yet been determined, but E4 is not required for transformation or episomal persistence of viral DNA, but interacts with the keratin networks and causes their collapse (Doorbar *et al.*, 1991). It has been suggested that E4 may have an important role in favoring and supporting the HPV genome amplification, besides regulating the

expression of late genes, controlling the virus maturation, and facilitating the release of virions (Logworth and Laimins, 2004). E4 also interacts with and disrupts the organization of intermediate filaments. The role of E4 in providing the release of virus is supported by the association of E4 with the cornified cell envelope (CCE), a highly resistant structure under the plasmatic membrane of differentiated keratinocytes in the stratum corneum. Furthermore, E4 may play role in regulating gene expression and has been shown to induce G2 arrest in a variety of cell types (Logworth and Laimins, 2004).

2.8.4 The E5 Protein

The E5 protein is a small hydrophobic peptide, approximately 83 amino acids in size that localizes primarily to the endoplasmic reticulum. When expressed alone, HPV E5 has weak oncogenic properties. But in tissue culture assays, HPV E5 can enhance the transforming activity of E6 and E7, suggesting that it may have a supportive role in tumor progression. The localization of E5 to the endoplasmic reticulum suggests its activity may be related to the trafficking of cytoplasmic membrane proteins through this cellular compartment. E5 has also been reported to alter the activity of the epidermal growth factor receptor (EGFR), in addition to reducing the surface levels of major histocompatibility complex (MHC) class I proteins, modulating the MAPK pathway and altering the levels of caveolin 1 (Moody and Laimins, 2010).

The E5 protein varies in length and primary amino acid sequence among the different papillomaviruses, but maintains its hydrophobic nature that promotes fusion between cells (Hu *et al.*, 2009). HPV16 E5 has all the characteristics of fusogenic proteins, including localization in plasma membrane, high level of hydrophobicity, and the ability for dimmers. Moreover, HPV16 E5 has been identified to be necessary and sufficient to induce cell-cell fusion with formation of tetraploid cell and cytokinesis failure (Hu *et al.*, 2009). The fusogenic activity of the HR-HPV

E5 protein contributes to fusion among cells generating aneuploidy with tetraploid cells and chromosomal instability. These events seem to precede and favor integration of HPV genomes, which in turn, leads to expression of viral-cellular fusion transcripts and further enhances expression of the E6-E7 genes, rendering transformed cells strong growth advantages (Zerfass *et al.*, 1995). Thus, the cell fusion HR-HPV E5-induced and cell cycle deregulation seems to have an important role in the early stages of the transformation process. This suggests that HR-HPV E5-induced cell fusion can be a critical event in the early stage of the development of HPV-associated cervical cancer (Gao and Zheng, 2010). As the E5 gene is frequently deleted in cervical cancers, it is believed that the E5 protein may play a role in the early stages of the process of cellular transformation, but is dispensable for the maintenance of malignant transformation (zur Hausen, 1996).

2.8.5 The E6 protein

The HPV E6 protein is formed by approximately 150 amino acids and contains two zinc-like fingers joined by an interdomain linker of 36 amino acids, flanked by short amino (N) and carboxy (C) terminal domains of variable lengths (Howie *et al.*, 2009). The best known property of the E6 proteins of HR-HPVs is the ability to bind and degrade the tumor suppressor protein p53, through the recruitment of the E6-associated protein (E6-AP), cellular E3 ligase that does not bind to p53 in the absence of E6. Both E6 proteins from HRHPV and LR-HPV bind to p53, but the interaction is stronger in HR-HPV (Lechner and Liamins, 2011).

The E6 protein can overcome the cell arrest and proapoptotic activities of p53 by targeting p53 for degradation, inactivating the Mdm2 pathway. E6 can also inhibit the transcriptional activities of p53 independently of E6-AP (Thomas *et al.*, 2005). Three different mechanisms have been proposed to explain this p53 inactivation: The first is inhibiting the binding of p53 to its target sequence in the genome; second, E6 may be able to inhibit p53 signaling by maintaining it in

cytoplasm; and third, the mechanism employed by E6 to inhibit p53 activity is the abrogation of the transactivation of p53 responsive genes via interaction with either the CBP/p300 or hADA3 histone acetyl transferases. The E6 proteins have been shown to bind to p300, and this interaction inhibits p35 acetylation at p53 dependent sites, leading to decreased expression from p53. However, unlike p300, E6 interaction with hADA3 results in hADA3 degradation (Lee *et al.*, 2007). E6 may also inhibit p53 activation by blocking the p14/ARF pathway. Thus, E6 is able to modulate transcription of p53-dependent genes by both degradation of p53 and by interaction with the p300 and hADA3 Trans activators (Shamanin *et al.*, 2008). The degradation or blocking of the p53 function inhibits apoptotic signaling that would eliminate the HPV infection cell. There are two major apoptotic pathways that can be triggered by different stresses: the extrinsic and intrinsic pathways. The E6 protein is able to disrupt both pathways to facilitate a cytoprotective environment and prevent cell death (Howie *et al.*, 2009). In addition, E6 is able to modulate transcription from other cellular signaling pathways as well as potentiating its ability to act as a diverse modulator of host cell signaling. It has been shown that E6 interact with three different proteins, such as a novel protein termed E6- targeted protein 1 (E6TP1) in an E6-AP dependent manner (Wright, 2006), beyond another protein with GAP activity, tuberin, that can also be bound and degraded by E6 (Zheng *et al.*, 2008). Furthermore, HR-HPV E6 has been shown to interact with two proteins that are part of the innate immune response to viral infection: interferon regulatory factor-3 (IFR-3) and toll-like receptor 9 (TLR9) (Hasan *et al.*, 2007). Exogenous expression of HPV16 E6/E7 has been shown to inhibit TLR9 transcription, leading to a functional loss of TLR9 signaling pathways within the cell (Hasan *et al.*, 2007).

The HR-HPV E6 is also able to interact with members of the PDZ family of proteins, promoting its proteasome-mediated degradation, an activity that seems to be required for induction of cervical cancer (Shai *et al.*, 2007). HR-HPV E6 PDZ binding can mediate suprabasal cell proliferation and this is thought to occur by uncoupling the cell proliferation and polarity control

that exist in a differentiated epithelium (Sterlinko *et al.*, 2004). LR-HPV E6 does not contain the PDZ-binding motif and therefore cannot target these proteins. Degradation of PDZ proteins results in cellular transformation due to loss of cell-cell contact and loss of cell polarity (Storrs and Silverstein, 2007). In addition, it has been demonstrated that the degradation of phosphatase PTPN13 by E6 results in anchorage-independent growth and a Ras-dependent invasive phenotype (Spanos *et al.*, 2008).

Another function of the HR-HPV E6 protein that is important for immortalization is their ability to activate the expression of the catalytic subunit of telomerase (hTERT). Thus, the E6 protein is able to promote the maintenance of the telomere, through the action of telomerase. Interestingly, over-expression of hTERT in conjunction with E7 is sufficient to immortalize human primary keratinocytes. The HPV E2 proteins are reported to repress hTERT promoter activity, but the interplay of E6 and E2 during the regulation of this promoter has not been investigated (Hamid *et al.*, 2009).

2.8.6 The E7 Protein

The E7 protein has around 100 amino acids in length and contains three conserved regions: CR1, CR2, and CR3 (Münger *et al.*, 2002). It will induce cellular proliferation by binding to several cellular factors. The best characterized of these interactions is with the RB tumor suppressor and the related family members p107 and p130. The binding of high-risk E7 to pRB disrupts the interaction between pRB and E2F, a family of transcription factors, resulting in the constitutive expression of E2F-responsive genes, such as cyclin A and cyclin E, and promotes premature S phase entry, DNA synthesis, and the progression of cell cycle (Zerfass *et al.*, 1995). Thus, in cells overexpressing the HPV E7 protein, this checkpoint control at G1/S transition is lost and the cells will continue their cell cycle, causing an uncontrolled cellular proliferation. Moreover, E7 induces the degradation of pRb via the proteasome-dependent pathway, using a mechanism that

involves association with and reprogramming of the cullin 2 ubiquitin ligase complex (Jo and Kim, 2005; Huh *et al.*, 2007). HPV E7 can also associate directly with cdk2/cyclin A and cyclin E complexes, resulting in an increased cdk2 activity (Nguyen & Münger, 2008). Another action of E7 that contributes to cellular immortalization is its interaction with the CDK inhibitors (CKI) p21 and p27, efficiently neutralizing their inhibitory effects on CDK2 activities, an important factor for G1 to S phase entry and progression (Moody & Laimins, 2010). The ability of E7 to inactivate these CKIs may contribute to its capacity to abrogate TGF- β mediated growth inhibition. Moreover, TGF- β also induces a cdk4/cdk6 specific CKI, P15Inkb, and p15Inkb-induced growth suppression, and these actions may require functional pRB, which is targeted for degradation by E7 (McLaughlin-Drubin and Münger, 2009). High-risk E7 has further been shown to increase the levels of the CDC25A phosphatase, which can induce tyrosine dephosphorylation of CDK2, promoting its activation (Moody and Laimins, 2010). E7 also affects the expression of S phase genes by directly interacting with E2F factors and with histone deacetylases (HDAC): E7-E2F6 interaction prevents repression of gene expression by E2F6, maintaining an S phase environment conducive for viral replication (McLaughlin-Drubin *et al.*, 2008), and E7-HDAC binding facilitates HDAC removal at promoters to activate transcription (Longworth & Laimins, 2004).

Another major apoptotic pathway targeted by HPV proteins is anoikis, a form of apoptosis that is triggered when normal cells attempt to divide in the absence of a matrix (Tasaki *et al.*, 2005). E6 and E7 interact with some factors involved with anoikis, such as paxillin, fibulin 1, and p600 (Huh *et al.*, 2005), promoting the prevention of anoikis. Furthermore, E6 and E7 interfere with the effects of various growth inhibitory cytokines that are induced following infection. High-risk HPV proteins repress the transcription of many IFN-inducible genes (Kanodia *et al.*, 2007, Tindle, 2002) and block apoptosis binding to TNF receptor 1, inhibiting the formation of the death-inducing signaling complex and consequent transduction of apoptotic signals (Filippova *et*

al., 2002). The exposure to E7 in a non-inflammatory epithelial environment can also be sufficient to induce a peripheral tolerance to E7 in the cytotoxic T lymphocytes population (Tindle, 2002). E6 also interacts with the adaptor protein FAS-associated protein with death domain (FADD) and caspase 8 to block cell death in response to FAS and TRAIL. Also, E6 can interfere with induction of the extrinsic and intrinsic (mitochondrial) apoptotic pathways through interactions with the pro-apoptotic Bcl2 members BAK and BAX, as well as by upregulation of the inhibitors of apoptosis such as the inhibitor of apoptosis protein 2 (IAP2, also known as BIRC2) and survivin (also known as BIRC5) (Garnett and Duerksen-Huges, 2006).

2.8.7 The L1 protein

The L1 gene corresponds to a sequence of about 1200 base pairs, which encodes a structural protein highly conserved among different HPV types, the (Xu *et al.*, 2006). The L1 protein is formed by five monomeric units of 55kDa that join to form a pentameric structure, totaling 72 per each capsid (Buck *et al.*, 2008). The L1 protein is highly immunogenic and has conformational epitopes that induce the production of neutralizing type-specific antibodies against the virus, which prevent the infection (Carter *et al.*, 2003), making it the target of prophylactic vaccines (Villa *et al.*, 2007; D'Andrilli *et al.*, 2010). Comparison among L1 sequences of different papillomaviruses suggests a conserved heparin-binding domain at the C-terminus, and the cleavage of this domain from L1 prevents binding to both heparin and human keratinocytes (Culp *et al.*, 2006; Selinka *et al.*, 2007). Thus, it is believed that the L1 major capsid protein contains the major determinant required for initial attachment of the viral particles to cell surface receptors, HSPGs, and therefore has an important role in infection (Schiller *et al.*, 2010).

2.8.8 L2 protein

L2 is a secondary component of viral capsid and it is present in a variable number of copies per

each capsid, being located on the inner surface in the central cavity below the pentamers of L1, where they are arranged to form the capsid (Buck *et al.*, 2008). Despite the paucity of L2 in the virion, this protein has recently been shown to have many more functions than a simple structural role. L2 contributes to the binding of virion in the cell receptor, favoring its uptake, transport to the nucleus, and delivery of viral DNA to replication centers. Besides, E2 helps the packaging of viral DNA into capsids and, due to the presence of a usual neutralization epitope in L2 proteins of many papillomaviruses; it may be instrumental in conferring immunity across different types of HPV. L2 also contributes to the interaction of virion in the cell surface. Two distinct regions in the N-terminal protein of L2 interact with the cell surface, and this interaction occurs after an initial low-specificity interaction between L1 and the cell surface. After this, a conformational switch occurs in the capsid, exposing the L2 epitopes and promoting interactions with a more specific secondary receptor. The cleavage of the N-terminus of L2 is necessary for the binding of L1 to the secondary receptor, an indication that L2 has an important role in HPV infection (Schiller *et al.*, 2010).

2.9 Protein Functions

The following viral proteins perform the functions below:

E1 protein performs the role of viral DNA replication, E2 controls viral transcription, DNA replication, and segregation of viral genomes, E4 proteins favor and support the HPV genome amplification, besides regulating the expression of late genes, controlling the virus maturation, and facilitating the release of the virions while the E5 protein Enhance the transforming activity of E6 and E7; Promotes fusion between cells, generating aneuploidy and chromosomal instability; Contribute to immune response evasion.

E6 Bind and degrade the tumor-suppressor protein p53, inhibiting apoptosis; Interact with proteins of the innate immune response, contributing to immune evasion and persistence of virus;

Activate the expression of telomerase.E7 Bind and degrade the tumor-suppressor protein pRB; Increase cdk activity; Affects the expression of S phase genes by directly interacting with E2F factors and with histone deacetylases; Induce a peripheral tolerance in cytotoxic T lymphocytes (CTL) and Down regulate the expression of TLR9, contributing to immune response evasion

L1 Major Capsid protein; contains the major determinant required for attachment to cell surface receptors. It is highly immunogenic and has conformational epitopes that induce the production of neutralizing type-specific antibodies against the virus.

L2 Minor capsid protein; L2 contributes to the binding of the virion in the cell receptor, favoring its uptake, transport to the nucleus, and delivery of viral DNA to replication centers. Besides, E2 helps the packaging of viral DNA into capsids.

2.10 Clinical Manifestations

It is believed as that after exposure to HPV, the virus infects the entire lower genital tract epithelium (Sh epherd and Bryson, 2008). The incubation period is highly variable, ranging from a few days to many years (20 to 30 years or more) (Sinal and Woods, 2005). When the infection process starts, there is a proliferation phase ranging from 3 to 6 months, when many lesions appear. After the response of B and T cells to the infection, what follows is the containment phase that also lasts for 3 to 6 months, when regression will occur to more than 80% of the lesions. The other 20% will have an active disease or recurrence after variable disease-free intervals (Franco and Steben, 2007). The clinical manifestations are variable and are associated with systemic and local immune response of each individual, with different environmental factors. Dependent on the host immune system, the course of the infection can take one of the three following forms. The most frequent is the Latent Infection, where no clinical manifestation of the infection occurs, and it is only detected by the HPV DNA detection methods. The second form is the Subclinical Infection with minimal clinical manifestation that is usually diagnosed by

colposcopy, cytology and histology. The third form which is the least common is the Clinical Infection. In this form there is an active expression of the disease, manifested mainly by genital warts, precancerous lesions and invasive cancer (Chow *et al.*, 2010).

The different manifestations are also dependent on different types of HPV (currently more than 200 types) and also the host immune system (Bernard, 2005). The low-risk HPV will mainly produce warts (condyloma) and the high risk HPV will mainly produce an intraepithelial lesion (Trofatter, 1997).

2.10.1 Clinical Lesions

Clinical lesions (only 2 to 3% of HPV infections) are mainly represented by condyloma acuminatum, Bowenoid papulosis (vulvar intraepithelial neoplasia usual type) and Buschke-Loewenstein tumor. The presence of acuminate lesions on the cervix are infrequent (in 6% of the women that have vulvar condyloma) and this represents an indication of high-risk HPV infections (20% of these infections have associated an intraepithelial lesion) (Scheurer *et al.*, 2005). Genital warts are easily recognized by papillary epithelial proliferations, often with vascular loops inside. Lesions may be single or multiple, scattered or confluent (Sadjadi *et al.*, 2003).

Vaginal warts can be detected by careful examination in more than one third of the cases of women who have vulvar warts. Generally, they are usually small and multiple and can be hidden by the speculum. The lesions may involve the entire length of the vagina, but most frequently occur in the upper and lower thirds of the vagina. Although vaginal warts are usually asymptomatic, vaginal discharging and itching can occur, and less often, post-coital bleeding, may be present ((Forcier and Musacchio, 2010).

The verrucous lesions in the vulvar region have increased in numbers in recent years, affecting

mainly younger women. Vulvar warts generally occur in moist areas of the skin and in places subjected mostly to trauma during intercourse. About 25% of the women with vulvar warts have these lesions in the anal and perianal region as well, and are not necessarily associated with the practice of anal sex. These lesions may be sessile or pedunculated, papular, hyperkeratotic or hyper pigmented.

Vulvar manifestations depend on each individual, ranging from small lesions to gigantic such as in the cases of a Buschke-Loewenstein tumor (Ambriz-Gonzalez *et al.*, 2005) Papular and hyperchromic vulvar lesions (vulvar intraepithelial neoplasia (VIN) usual type) represent today, a high-grade intraepithelial neoplasia (Forcier and Musacchio, 2010). Approximately 18% of women with vulvar condyloma have anal and perianal warts as well. Depending on the extent of the injuries, discomfort or bleeding may occur during evacuation. In these cases, the rectal examination should also be performed, since approximately 10% of women with anal warts, exhibit rectal lesions (Nadal *et al.*, 1999).

When evaluating male partners of women with genital disease associated with HPV, approximately 40 to 50% of them also have lesions. The lesions may manifest as penile warts, papules or papillae. The lesions appear mainly in areas of trauma, especially related to sexual activity (the penile shaft, preputial cavity, coronal sulcus and glans).

Urethral involvement is more frequent in men than women (10 to 28% of the men with genital warts and less than 5% of the women with genital diseases associated with HPV) (Buechner, 2002). Genital warts at the base of the penile Condyloma in the urethra of a young man

2.10.2 Sub-clinical Lesions

Subclinical lesions represent 60% of the cases of external anogenital HPV and 95% of the cases of cervical HPV infection. The main symptoms are micropapillary, micropapular, spike, and

keratotic lesions. The diagnosis of these lesions is accomplished primarily by colposcopy, cytology and histology (Forcier and Musacchio, 2010). The cytopathic effects of HPV infection, specifically koilocytotic atypia, dyskeratosis and cellular multinucleation are detected in 2 to 3% of routine Pap smears). The cytological and histological patterns of HPV-induced lesions are essentially the same (Wright, 2006).

Cervical intraepithelial neoplasia (CIN) is the most common manifestation of HPV in the cervix. These lesions are manifested by colposcopy using acetic acid that produces acetowhite epithelium, punctation or mosaic. At the colposcopic exam, the cellular changes in the low grade squamous intraepithelial lesions (LSIL) are discrete while in high-grade lesions (HSIL) are bigger, including an atypical vascularization. An aceto-white lesion outside the cervical transformation zone is highly suggestive of an HPV infection. The low-grade lesions often regress spontaneously, ranging from 25 to 60% in one year. The regression rate for high-grade lesions is much smaller (Chase *et al.*, 2008).

The colposcopic examination of the vulva after the application of acetic acid at 5% permits the identification of the minimum changes associated with HPV, usually expressed by the acetowhite epithelium. These changes are often multifocal and commonly involve the vaginal fourchette and labia minora. It is necessary, however, to have an expert colposcopist to differentiate the subclinical alterations induced by HPV from inflammatory changes (Gagné, 2008). The vulvar intraepithelial neoplasia (VIN) associated with HPV (VIN usual type) has a very small risk of progression to an invasive lesion. The most severe intraepithelial lesions (VIN differentiated type, that are not associated with HPV) tend to be multicentric and multifocal. These lesions may be associated with pruritus and local irritation (Heller, 2007).

Subclinical changes in the perianal and anal area are much less frequent and practically all are associated with an aceto-white epithelium of varying severity after the use of acetic acid at 5%

(Chin-Hong and Palefsky, 2002).

2.11 Immunology of Human Papillomavirus

Human papillomavirus protects itself against the host immune response by the following strategy

2.11.1 Evading Human Immune Response

HPV infection is restricted to epithelial cells. In the genital tract, specifically both keratinizing (skin) and non-keratinizing (mucosa) stratified squamous epithelia may be infected. The stratified epithelia are composed of undifferentiated basal and Para basal cells that have the ability to proliferate (Doobar, 2001), and differentiating superficial layers. The cells in the most superficial layers are fully differentiated end-stage cells that have lost their ability to replicate and are shed into the environment (Munoz *et al.*, 2006; Hoffman *et al.*, 2006). HPV multiplication is intimately linked with the differentiation of the stratified squamous epithelium and it is for this reason that HPV are not cultivatable by traditional viral culture methods.

In natural infection, HPVs cause infection following a minor abrasion or break of the squamous epithelium, firstly binding to the basement membrane. This complex interaction results in conformational change of the L1 epitope, before HPV enters the keratinocyte by a novel endocytic pathway. The L1 portion of the HPV virion protein coat binds to heparin sulfate proteoglycans on the basal cells, which appear to be the primary attachment factor (Roberts *et al.*, 2004; Culp *et al.*, 2006 and Day *et al.*, 2008). However, the steps leading to virion internalization are not completely understood. In a murine challenge model, it appears the capsids undergo a conformational change while bound to the basement membrane that results in L2 cleavage, followed by the exposure of an N-terminal cross-neutralization L2 epitope and

transfer of the capsids to the epithelial surface (Kimes *et al.*, 2009). Basal cells are also relatively accessible in transformation zones (TZ) where multi-layered squamous epithelia meet simple glandular epithelia (Mosciski *et al.*, 2006). This is the same region where squamous metaplasia occurs (a process whereby glandular epithelium is replaced by squamous). As a result of this junction, and the metaplastic process, immature basal cells are accessible in the TZ (Joyce *et al.*, 1999; Giroglou *et al.*, 2001; Shaft Kerant *et al.*, 2003).

After infecting basal cells, HPV undergoes a low-level replication to about 100 copies of viral DNA per cell (Munoz *et al.*, 2006). As the cells undergo normal differentiation and migrate towards the epithelial surface, viral DNA replication is upregulated resulting in several thousands of copies of HPV DNA per cell (Hoffman *et al.*, 2006). This high-level replication is dependent on host-cell replication enzymes but is mediated by HPV proteins E1/E2 as well as E6/E7 (DImajio and Liao 2006). The HPV E1 protein is a DNA helicase that binds to the viral orf and unwinds the double-stranded DNA (Seo *et al.*, 1993; Yang *et al.*, 1993). The E2 protein both regulates viral expression and binds to the E1 protein, increasing the binding affinity of the E1/E2 complex to the origin of viral replication (Frattinni *et al.*, 1994). E6 and E7 prolong the lifespan of the host replication enzymes (Middleton *et al.*, 2003). Although incident infection may be entirely undetected, productive infections of the cervix results in lesions detected as low-grade squamous intraepithelial lesions (LSIL), or equivocal Pap tests are actually the viral cytopathic manifestations of incident HPV infection. On biopsy, these lesions are recognized as CIN 1. The cyto-histologic feature of koilocytosis is characteristic, but not diagnostic, of productive HPV infection. These lesions are likely to be cleared as a result of cell-mediated immune responses directed to HPV proteins (Stanley, 2008). These responses are eventually followed by antibody generation to HPV L1 in approximately half of those in whom an HPV DNA is detected. This is a slow and generally weak response to L1 and many women do not seroconvert (Carter *et al.*, 2000).

2.11.2 Host Immune Response to HPV Infection

Human Papillomavirus persistence, and the increased risk for neoplastic progression, is facilitated by an insufficient initial or sustained anti-HPV immune response and the capacity of HPV to evade natural host immune responses (HO *et al.*, 2004; Stern, 2005). Unlike blood borne pathogens, such as hepatitis B virus, which induce systemic immune responses, HPV has no viremic phase, as infection is restricted to the epithelial compartment. Therefore, an immune response to HPV needs to be initiated at the site of infection in mucosa. As HPV infection is not cytolytic, the innate immune responses that would normally occur in response to cell death are limited and the virus is shielded from circulating immune cells as infection becomes established. Also, viral proteins are not expressed at high levels until the later stages of viral life-cycle when end-stage differentiated epithelial cells are shed from the epithelial surface. Together, the limited innate immune response, the low levels of viral gene expression in the lower layers of the epithelium, and the lack of cell death or necrosis, generally result in a delayed adaptive immune response to initial papillomavirus infection. By administering HPV vaccines parenterally, the L1 is taken up to regional lymph nodes and hence many of these HPV immune evasion mechanisms that naturally occur are overcome. (Ho *et al.*, 2004)

Natural humoral immune responses to HPV infection are weak, in part due to HPV evasion mechanisms and the lack of viremic phase during early infection. However, most early HPV infections will resolve spontaneously. Humoral immune responses begin with the growth and maturation of B cells which are dependent upon interaction with antigen presenting cells (APCs) and the cytokine profile secreted by T-helper cells. The resultant antibodies function to neutralize and opsonize foreign antigen for destruction, preventing infection of susceptible host cells. (Doobar, 2005).

2.11.3 Innate Immunity against HPV Infection

The intraepithelial life-cycle of HPV evades immune recognition, in part through its effects on Langerhans cells and dendritic cells. One of HPV's adaptive evasion mechanisms is its ability to replicate without inducing cell death and subsequent pro-inflammatory signals after apoptosis (Stanley, 2006). In the non-inflammatory environment of an incident HPV infection, APCs such as macrophages and Langerhans cells are relatively ineffective (Stanley, 2008). This probably results in partially-activated dendritic cells with a limited ability to migrate to the loco-regional lymph nodes. An antigen-specific immune response towards a pathogen may not be initiated by lymphocytes, due to immunologic tolerance which occurs when there is incomplete activation of APCs. Tolerized lymphocytes may not be able to respond to a subsequent antigen exposure, even in an activating environment. A second evasion mechanism is by suboptimal Langerhans cell responses as a result of low-level expression of HPV E6 and E7 and other viral proteins within the basal and immediate suprabasal layers of the stratified squamous epithelium, which are under active immune surveillance by Langerhans cells (Doobar, 2005). Production of virions occurs only in the more immune-privileged differentiated apical layers of the epithelium. In the HPV-infected transformation zone (where the squamous epithelium and columnar epithelium meet) undergoing neoplastic transformation, especially in high grade CIN, there is both a reduction in numbers and a change in phenotype of Langerhans cells (Gianni *et al.*, 2002; Hubert *et al.*, 2005). Interactions between HPV and Langerhans cells are likely to be advantageous for viral persistence (Einstein *et al.*, 2009).

2.12 Diagnosis

The conventional methods of viral diagnosis such as electron microscopy, cell culture, and certain immunological methods are not suitable for HPV detection. HPV cannot be cultured in cell cultures. The important methods to diagnose HPV infection are: Colposcopy and acetic acid test, Biopsy, DNA test (PCR, Southern Blot Hybridization, In Situ Hybridization) and Pap smear

2.12.1 Colposcopy

Colposcopy is a procedure performed by specially trained clinicians as an outpatient procedure using a low-powered microscope, the colposcope. (Chuang *et al.*, 2010) Colposcopy is the examination of the cervix, vagina, and in some instances the vulva after the application of acetic acid solution; coupled with obtaining colposcopically directed biopsies of all lesions suspected of representing neoplasia. (NHS, 2011) Colposcopic findings are graded according to degree of acetowhite lesion, surface contour, mosaic pattern, and punctuation. Greater abnormalities of these parameters are related to severity of the lesions.

2.12.2 Acetic Acid Test

Soaking acetic acid into suspicious lesions can enhance the degree of suspicion in lesions without classic features.

The method involves applying a 3–5% acetic acid–moistened gauze pad for 5-10 minutes on suspected lesions of the penis, cervix, labia, or perianal area.

Inconspicuous, flat, genital lesions that might be difficult to assess become visible. Genital warts, dysplastic, and neoplastic tissues turn white (acetowhite).

False-positive results are common and can result from anything that causes parakeratosis (e.g., candidiasis, psoriasis, lichen planus, healing epithelium, sebaceous glands).

The acetic acid test should not be used for routine screening.

It can be used for visualizing subclinical genital HPV-associated lesions, identifying lesions for target biopsy, and for demarcating lesions during surgical therapy.(Martin *et al.*, 2011)

2.12.3 Biopsy

Colposcopy allows tissue sampling (biopsy) that is targeted to the abnormal areas. In fact, the

biopsy of abnormal areas is a critical part of colposcopy because treatment will depend on how severe the abnormality is on the biopsy sample. If the biopsy results show pre-cancer (dysplasia) or cancer, then treatment is recommended. The dysplasia may be mild, moderate, or severe (CDC, 2016).

Excisional biopsy is recommended when colposcopic appearances indicate high grade abnormality, when low grade colposcopic change is associated with severe dyskaryosis or worse, or when a lesion extends into the canal. (Chuang *et al.*, 2014)

In genital warts, the most characteristic feature is the presence of koilocytes, which are mature squamous cells with clear perinuclear zone. The nuclei of koilocytes may be enlarged and hyperchromatic, double nuclei are seen often as well. (CDC, 2014)

2.12.4 DNA Techniques

Initial methods of HPV detection used were direct probe hybridization such as dot blot and Southern blot. Besides being labor-intensive and time consuming, they had low sensitivity, required large amounts of DNA in clinical samples, and have largely been superseded by amplification technology, which has allowed detection of low-level virus copy numbers in clinical samples. The established routine method for viral detection is the hybridization of viral nucleic acids. The two main DNA techniques are highlighted below

2.12.4.1 Hybrid Capture HPV DNA Test 2 (hc2)

Hybrid capture HPV DNA test 2 in conjunction with the Pap test is now approved by the FDA (Madelblatt *et al.*, 2002). Since the FDA-approved Hybrid Capture 2 test can detect as little as 1 pg of HPV DNA/ml; its sensitivity and specificity are almost comparable with PCR-based detection methods. The advantages of the Hybrid Capture 2 test are the relatively simple handling and good reproducibility of results, which make this test the best standardized HPV

detection method. While the exact HPV type cannot be identified, “low-risk” (Chuang *et al.*, 2010; NHS, 2011) and “high-risk” (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) HPV genotype groups (HR HPV and LR HPV) are detected.

2.12.4.2 Polymerase Chain Reaction

PCR is a selective target amplification assay capable of exponential and reproducible increase in the HPV sequences present in biological specimens. The amplification process can theoretically produce one billion copies from a single double stranded DNA molecule after 30 cycles of amplification. (Garland *et al.*, 2011)

Indications: Primary Screening in Conjunction with the Papanicolaou (Pap) and the Test or as a Stand-Alone Test for women over 30 years of age

2.12.5 PAP Smear

It is a screening test first described by Papanicolaou and Traut. Apart from premalignant and malignant changes, viral infections like HPV infection and Herpes can also be detected. Positive test requires further confirmatory tests like coloscopy, cervical biopsy, and DNA tests like PCR.

Patient is placed in dorsal position and cervix is exposed with Cusco's speculum taking scraping from squamocolumnar junction with the help of Ayre's spatula by rotating it all around. The scrapings are spread on glass slide fixing it with 95% ethyl alcohol and ether, or fixative spray (cytospray). For cytological evaluation, scrapings are taken from upper lateral part of vaginal wall.

Women from ages 21 to 30 be screened every two years instead of annually, using either the standard Pap or liquid-based cytology. Women age 30 and older who have had three consecutive negative cervical cytology test results may be screened once every three years with either the Pap or liquid-based cytology. Women with certain risk factors may need more frequent screening,

including those who have HIV, are immunosuppressed, were exposed to diethylstilbestrol (DES) in utero, and have been treated for cervical intraepithelial neoplasia (CIN) 2, CIN 3, or cervical cancer.

2.13 Epidemiology of Human Papillomavirus

Approximately 6.2 million new HPV infections occur every year in the United States, and approximately 20 million individuals are currently infected (Munoz *et al.*, 2003). HPV is spread by skin-to-skin sexual contact and is prevalent in all sexually active populations. The Centers for Disease Control estimates that at least half of all sexually active individuals will acquire HPV at some point in their lives, whereas at least 80% of women will acquire an HPV infection by age 50 (CDC,2014). HPV 16 alone is linked to more than 50% of all cervical cancers (CDC, 2014); thus, the prevalence of HPV 16 is of special interest.

One study utilized an experimental serological test to determine the presence of antibodies to HPV 16, which signify prior exposure to HPV, instead of the more commonly assessed viral DNA which is indicative of active infection. Women were more likely to be seropositive for HPV 16 (17.9%) than were men (7.9%). However, this methodology may in fact underestimate the true prior exposure to HPV 16, because <60% of women infected with HPV 16 develop type-specific antibodies (Stone *et al.*, 2002)

The human papillomavirus (HPV) infection is the most common sexually transmitted diseases (STD) worldwide, representing a significant health problem due to its high prevalence and transmissibility (Trim *et al.*, 2012). It is estimated that 75 percent of the sexually active population has been exposed to HPV (Koutsky, 1997). Prevalence estimate vary according to the diagnostic method and the population examined, with higher rates being observed in studies using molecular biology and including young women with high- risk sexual behavior (Trottier and Franco, 1996). Virtually all cases of cervical cancer worldwide are caused by persistent

infection with one or more of approximately a dozen carcinogenic genotypes of human papillomavirus (Schiffman *et al.*, 1993; Walboomer *et al.*, 1999; Munoz *et al.*, 2003)

In a recent meta-analysis, global HPV prevalence in North America and Europe was estimated at 21% with sub Saharan Africa topping the list at 24% (Dahlstrom *et al.*, 2010; Ezenwa *et al.*, 2013).

In Nigeria, the prevalence of HPV is high in all female groups and highest in women aged 15-23yrs (Ezenwa *et al.*, 2013; Bruni *et al.*, 2014). Two recent sources have provided estimates that may reflect global prevalence, age-specific prevalence, and type-specific prevalence, along with international variability of HPV. A multicenter; centrally coordinated international study conducted by the International Agency for Research on Cancer (IARC) has provided data from 15 areas in 4 continents regarding women aged from 15 to 74 years. The age-standardized prevalence ranged from less than 5% in some Mediterranean and South East Asian countries to more than 15% in several countries in Latin America and among a few African populations (IARC, 2014).

In a comprehensive review of studies that used standardized inclusion criteria and controlled for variables that may have challenged the comparability of the studies, prevalence estimates of HPV infection among women with The epidemiology of human papillomavirus infection and its association with cervical cancer S9 negative cytologic results ranged from 10% to 15%. Age-specific prevalence estimates showed HPV DNA to be more prevalent among very young women, with a decline in young adult women and a variable pattern afterwards. In some countries, notably in the Americas, the prevalence increased again in postmenopausal age groups. In Europe, a plateau in the middle-age groups was maintained whereas in other high-prevalence countries in Asia and Africa the prevalence remained fairly constant across all age groups (Winer *et al.*, 2003).

2.14 Behavioral determinants of HPV infection

Epidemiological studies investigating risk factors for HPV infection have clearly and consistently shown that the key determinants of infection in women are the number of sexual partners; the age at which sexual intercourse was initiated; and the likelihood that at least 1 sexual partner was an HPV carrier as estimated by his sexual behavior patterns (Brown *et al.*, 2005). The role of men in HPV infection of women was investigated in early epidemiological studies using questionnaires that addressed the sexual behavior of the husbands or sexual partners of women with and without cervical cancer. More recent studies had, in addition, been able to detect the presence of HPV DNA in exfoliated cells from the penile shaft, the coronal sulcus, and the distal urethra (Longworth and Liamins 2004).

These and other studies have established that the risk of cervical cancer for a given woman is predictable from the sexual behavior of her husband or sexual partner as much as from her own sexual behavior. In populations where female monogamy is predominant, female sex workers play an important role in the maintenance and transmission of HPV infections. Moreover, the probability that a woman is an HPV carrier, as well as her risk of developing cervical cancer, have been shown to be related to the presence of HPV DNA on the penis or in the urethra of her husband or sexual partner (Stone *et al.*, 2002). These observations confirmed a scientific hypothesis formulated almost 30 years ago that male sexual behavior is a central determinant of the incidence of cervical cancer.

2.15 Treatments

The treatments available for HPV infection are the topical therapy, lazer therapy, imiquimod therapy, interferon therapy as discussed below

2.15.1 Topical Therapy

Biochloroacetic acid (BCA) or trichloroacetic acid (TCA) are desiccant acids that are absorbed by the treated tissue. They are especially effective on the moist lesions of mucous membranes, whose water content is high. These acids should be applied directly to the wart, preferably with magnification, to allow precise placement on small lesions and avoid healthy skin. The depth of injury can be limited by close observation of the intensity of whiteness of the treated area. A burning sensation occurs for 5 to 15 minutes, but it may be avoided by spraying a topical anesthetic before treatment. The desiccant acids are not toxic and can be used safely during pregnancy or inside the vagina (Yang *et al.*, 2009). For intravaginal or cervical condylomas, the acid is applied with colposcopic guidance and allowed to dry for 2 minutes before a saline-soaked swab is used to remove the remaining acid. It is not advisable to treat the entire vagina because significant sloughing could occur and lead to stricture. The patient should be treated weekly until all visible lesions are gone.

The other popular topical agent for the treatment of cervical condylomas is podophyllin. Its biologic activity is due to an antimetabolic effect, which leads to sloughing of the tissues treated. Unlike with the desiccant acids, its maximum effect takes place several days after application. Podophyllin may have systemic neurologic and bone marrow toxicity when used over a large, moist skin surface, and therefore cannot be used during pregnancy or to treat vaginal condylomas. Since a 25% podophyllin resin contains between 50 and 100 mg/mL of the active agent podophyllotoxin (Hellberg *et al.*, 1995), variable efficacy and toxicity occur with this method of treatment. However, a 0.5% podophyllotoxin solution is now available for the treatment of condylomas.

The patient applies the solution to warts twice daily for 3 days, followed by 4 days without treatment. Complete clearance occurs in approximately 50% of women after 4 treatment cycles (Hellberg *et al.*, 1995). Topical therapy will initially clear most condylomas, but primary therapy

failures and secondary recurrences lead to an overall clearance rate of approximately 50%. Subsequent treatment strategies depend on the appearance of the lesions. The thick, chronic, keratinized warts are best treated with tissue-destructive methods and adjuvant interferon therapy ((Yang *et al.*, 2009). Extensive, diffuse warts that regrow between each treatment visit are best treated with imiquinod. When condylomas are unresponsive to treatment, biopsy is important to exclude an underlying malignancy such as verrucous carcinoma.

2.15.2 Laser Therapy

The laser of choice is the carbon dioxide laser. It is attached to the colposcope, so that the surgeon may identify the dermatologic surgical planes. This technique allows for tissue destruction, which occurs from both immediate vaporization and delayed tissue necrosis, to be confined to the epidermis and superficial papillary dermis (Duus *et al.*, 1995). Power density should be set above 750 watts/cm³ to reduce the delayed tissue necrosis, which results from heat buildup. Whenever possible, outpatient treatment with local anesthesia is preferred. Since the wart is an epidermal growth, only the epidermal layer needs to be removed. During the laser procedure, the vaporized debris is frequently wiped away with wet gauze so that the classic appearance of the papillary dermis can be viewed. The results of laser treatment alone are extremely variable and depend on patient selection, number of treatments used, and volume of skin treated. Initially, cure rates higher than 90% were reported (Agnaud *et al.*, 2008), but further follow-up revealed long-term control to be only 67 % (Duus *et al.*, 1995).

For large exophytic lesions, the electrodiathermy loop excision procedure is gaining popularity. Precise control of the depth of destruction is important so that scarring does not result. Electrosurgical excision results in a small amount of thermal damage. Electrocautery or cryocautery, on the other hand, result in thermal injury and lack of control of tissue damage, which lead to a higher risk of scar formation. The efficacy of the electrodiathermy loop

excisionis not known; however, if used in conjunction with the colposcope to identify and destroy all the lesions, it should achieve results similar to those of laser therapy. Reports from the literature on electrocautery and cryocautery techniques reveal a control rate of 40% to 55% (Christensen *et al.*, 2005).

2.15.3 Imiquimod Therapy

Imiquimod is a topical 0.5% cream that the patient applies weekly for up to 16 weeks. It acts as an immune response modifier with the ability to induce the production of interferon- α , tumor necrosis factor, and various other cytokines. The phase 3 clinical trials of imiquimod have shown a 72% clearance rate, vs. a 20% rate in patients treated with placebo. The adverse effects of imiquimod are local skin reaction, with up to 62% of patients reporting some redness or erythema and 5% reporting ulceration (Abdullah *et al.*, 1993). Only 1% of patients discontinued the medication because of severe local skin reaction. It is not only the local efficacy of imiquimod that is important; it is also the low recurrence rate of approximately 13% in the 3 months of follow-up after treatment. The low recurrence rate is a result of induced immunologic memory to HPV (Langley, 2010).

Some treatments have been partially efficacious and are still in widespread use and acceptance today to treat anogenital disease. Notably among these are podophyllotoxin (isolated from the May apple) and a formulated version called podophyllin or Condylox, which have been used to suppress genital warts (Diamantis *et al.*, 2009). One of the major problems is that podophyllotoxin is anti-mitotic and does not readily allow for the re-epithelialization of healthy skin to replace the erosion caused by its application. Imiquimod (Aldara), an immune response modifier, has been used to treat genital lesions with good results (Garland *et al.*, 2006)

2.15.4 Interferon Therapy

Injections of interferon alfa-2 into the wart-bearing skin 3 times weekly for 3 weeks have resulted approximately in a 50% disappearance of the treated warts, vs. 15% in placebo groups (Monesenego *et al.*, 1996). The need to inject the interferon and the adverse effects of Clinical manifestations of HPV infection S35 fever, muscle aches, and flu-like symptoms have limited its use to patients in whom other therapies have failed. Systemic injection of interferon has been used successfully in some of the patients but relapse can occur after discontinuation of treatment. (Bornstein *et al.*, 1997). A single study reported that treating cervical dysplasia patients with celecoxib, a selective COX-2 inhibitor, prevented disease progression and increased regression (Yang *et al.*, 2009). Building on the wealth, breadth, and depth of knowledge gained in the past decades of intensive worldwide research on papillomaviruses, selective inhibitors of different pathways essential to viral replication and long-term persistence are being sought. Diindoylmethane (DIM) have received well-deserved attention because of their ability to suppress laryngeal papillomas in some patients, possibly by diminishing the production of pro-estrogenic metabolites in favor of anti-estrogenic derivatives (Basita *et al.*, 2010) Artemisinin, artesunate, and related compounds are antimalarial agents derived from wormwood. They are cytotoxic against HPV-immortalized cells or cervical carcinoma cells, but not against normal cervical cells.

2.16 Prevention and Control

Transmission of HPV can be reduced but not eliminated with the use of physical barriers such as condoms (CDC, 2014). Recent studies demonstrated a significant reduction in HPV infection among young women after initiation of sexual activity when their partners used condoms consistently and correctly (ACOG, 2010). Abstaining from sexual activity (i.e., refraining from any genital contact with another individual) is the surest way to prevent genital HPV infection. For those who choose to be sexually active, a monogamous relationship with an uninfected

partner is the strategy most likely to prevent future genital HPV infections (CDC, 2010).

2.16.1 Cervical Cancer Screening

Most cases and deaths from cervical cancer can be prevented through detection of precancerous changes within the cervix by cervical cytology using the Pap test. Currently available Pap test screening can be done by a conventional Pap or a liquid-based cytology (Schiller, 2012). CDC does not issue recommendations for cervical cancer screening, but various professional groups have published recommendations (Sulslow, 2012)

Cervical cancer screening recommendations were revised in 2012 after the U.S. Preventive Services Task Force (USPSTF) and a multidisciplinary group, including the American Cancer Society (ASC), American Society for Colposcopy and Cervical Pathology (ASCCP), and the American Society for Clinical Pathology (ASCP) reviewed new evidence. Previously, recommendations varied by organization. Since 2012, all organizations have recommended that screening should begin at age 21 years. While there are slight differences in other aspects of the recommendations, all groups recommend screening in women aged 21 to 65 years with cytology (Pap test) every 3 years. For women aged 30 to 65 years who want to lengthen the screening interval, screening can be done with a combination of cytology and HPV testing (“co-testing”) every 5 years (Schiller, 2012). The use of HPV vaccine does not eliminate the need for continued Pap test screening, since 30% of cervical cancers caused by HPV types are not included in the vaccines available (CDC, 2011).

2.16.2 Prophylactic HPV Vaccines

Available prophylactic HPV vaccines and mechanism of action: There are two vaccines currently licensed that are sold internationally. Both are prepared from purified L1 protein, the major capsid protein that self-assembles to form type-specific HPV virus-like particles (VLPs). These

VLPs closely resemble the outer surface of HPV virions. VLPs contain no viral DNA and are therefore non-infectious. The vaccines are designed for prophylactic (preventative) use and have not been found to effectively clear existing HPV infections or treat HPV-related diseases (Ault *et al.*, 2007; Hildesheim, 2007).

The quadrivalent vaccine was first licensed in the United States in 2006. The L1 proteins for each type are expressed via a recombinant *Saccharomyces pombe* (type of yeast) vector. Each 0.5 ml dose contains 20 µg of HPV-6 L1 protein, 40 µg of HPV-11 L1 protein, 40 µg of HPV-16 L1 protein and 20 µg of HPV-18 L1 protein adsorbed onto 225 µg of the adjuvant, amorphous aluminium hydroxyphosphate sulfate (AAHS). This vaccine has been licensed for use in girls as young as age nine, to prevent cervical precancers, cervical cancers, vulvar precancers and vaginal precancers, as well as anogenital warts. In some countries, the vaccine is also licensed for the prevention of anogenital warts in males (Satterwhite *et al.*, 2013).

The bivalent vaccine was first licensed in 2007. The L1 proteins for each type are expressed via a recombinant baculovirus (type of insect cell) vector. Each 0.5 ml dose contains 20 µg of HPV-16 L1 protein and 20 µg of HPV-18 L1 protein adsorbed onto a proprietary AS04 adjuvant system containing 500 µg of aluminium hydroxide and 50 µg of 3-O-desacyl-4'-monophosphoryl lipid A, a novel adjuvant. This vaccine has been licensed for use in girls as young as age 10 to prevent cervical precancers and cervical cancers. Registration for indications in males has not been sought (Moyer, 2012).

The mechanisms of action of the HPV L1 vaccines are not known. Current hypotheses are based on data from experiments in rabbits (Brectburd *et al.*, 1995) and dogs (Ghim *et al.*, 2000) demonstrating that naive animals passively immunized with purified serum IgG from either VLP immunized or naturally-infected animals were completely protected against high dose of viral challenge. Briefly, it is thought that VLPs are rapidly bound by myeloid DCs and B lymphocytes

and signal via TLR-dependant pathways essential for B-cell activation and antibody generation (Stanley 2008; Yang *et al.*, 2000; Yang *et al.*, 2005). The protection is thought to be due to direct action of serum antibodies transudating and exudating to the site of infection at the cervix. However, the level of antibody required for protection is not known.

2.16.2.1 Adjuvants in HPV Vaccines and Clinical Relevance

HPV L1 VLPs are themselves highly immunogenic. However, adjuvants in HPV L1 vaccines increase VLP immunogenicity by inducing cytokines or chemokines, which then act directly or indirectly on helper lymphocytes to modulate immune responses. Adjuvants also increase both antigen uptake by, and maturation of, antigen-presenting cells (Vogel *et al.*, 2005). It is believed that the adjuvants in HPV L1 vaccines: 1) accelerate the generation of robust immune responses; 2) induce local mucosal immune responses; 3) generate antibodies with enhanced avidity and neutralizing capacity; 4) elicit the response of cytotoxic T cells; 5) increase the response rate in low-responder individuals; 6) reduce the required amount of antigen necessary to generate the desired immune response (Pashine *et al.*, 2005).

Saccharomyces cerevisiae, and eucaryotic insect cells when infected with baculovirus expression vectors. The requirement for maintaining native conformation adds considerably to the difficulty of manufacture, storage, and delivery of vaccines. As the L1-based VLPs are largely HPV type restricted and expensive to produce, additional approaches are being proposed. One is to use capsomeres assembled from five L1 monomers. The antibodies elicited are also type specific but the antigen can be produced rather inexpensively in bacteria (Castle *et al.*, 2004; Einstein *et al.*, 2009).

Another approach is to develop a cross-reactive vaccine. A region of L2 is relatively conserved

among diverse papillomaviruses, and a peptide corresponding to that region indeed elicits broadly cross-reactive antibodies in preclinical studies (Einstein, 2009).

One current approach uses DNA-based vaccines to express a detoxified E7 protein, which no longer can bind the pRB family of pocket proteins. This has been efficacious in preclinical studies. A few clinical trials are ongoing (Jourg *et al.*, 2008). The most common practice is to remove the lesions using a variety of surgical procedures including cold knife, microdebrider, various types of laser, cryosurgery, and electrocautery, according to the anatomic site. A number of small molecules have also been used either alone or as adjunctive therapies, as described below. Small molecule drugs to treat genital warts are available. Topically delivered potions have been used to treat papillomavirus infections from time immemorial, just as traditional medicines derived from natural products have been used against many other common ailments. Some treatments have been partially efficacious and are still in widespread use and acceptance today to treat anogenital disease. Notably among these are podophyllotoxin (isolated from the May apple) and a formulated version called podophyllin or Condylox, which have been used to suppress genital warts (Giuliano *et al.*, 2007). One of the major problems is that podophyllotoxin is anti-mitotic and does not readily allow for the re-epithelialization of healthy skin to replace the erosion caused by its application.

2.16.3 Small Molecule Drugs to treat RRP

In attempts to slow down or to curb post-surgical regrowth of airway papillomas, various adjunctive pharmacotherapies and immunotherapies have been attempted. The persistent clinical dilemmas are that (i) none of the agents used to treat HPV infections, including those described below, is reliably effective in all patients and (ii) collateral damage to normal host epithelium can be considerable. Building on the wealth, breadth, and depth of knowledge gained in the past decades of intensive worldwide research on papillomaviruses, selective inhibitors of different

pathways essential to viral replication and long-term persistence are being sought (Slade et al; 2009). As almost all laryngeal papillomas are caused by HPV-6 or HPV- 11, clinical application of future type-specific inhibitors would be a possibility. However, in almost all other anatomic sites, HPV type-specific agents are clinically impractical because of the large number of possible genotypes responsible for the lesions.(WHO, 2009) Vitamin A (all-trans-retinoic acid) treatment prevents keratinocyte differentiation, which of course then blocks much of the HPV reproductive program. However, this also prevents full healing, leaving incompletely differentiated squamous epithelia subject to painful sores and possible microbial infections. (Stanley, 2008)

Indole-3-carbinol and its derivatives such as diindolylmethane (DIM) have received well deserved attention because of their ability to suppress laryngeal papillomas in some patients, possibly by diminishing the production of pro-estrogenic metabolites in favor of anti-estrogenic derivatives, (Wheeler *et al*; 2008; WHO, 2009). I3C is naturally conjugated to several possible sugars as a gluconate in many cruciferous vegetables. In the acidic environment of the stomach, I3C is a very reactive compound and, among other possibilities, can dimerize to DIM. The active agent can also be chemically prepared as DIM, minimizing unpredictable reactions after ingestion. The proposed mode of activity is that I3C and DIM have structural similarities to estrogen (b-estradiol).

The natural elimination pathway for any hydrophobic steroid hormone involves hydroxylation to increase solubility. B-estradiol is subject to conversion either to 16- α -hydroxy estrone (a strong pro-estrogenic derivative) or 2-hydroxy estrone (an anti-estrogenic derivative). (CDC, 2010) Speculation is that the cytochrome P450 hydroxylase induced by I3C and DIM to make these aromatic compounds more aqueous soluble happens collaterally to convert b-estradiol to the 2-hydroxyestone and ultimately to suppress mitogenic stimulation to papillomas. Many RRP patients benefit from I3C or DIM intake on a daily regimen and, while consistent use can

prolong intervals between surgeries, only rarely is this sufficient for complete remission. Interferon- α generally blocks viral reproduction and is an integral part of natural anti-viral defenses in mammals. (Bruns *et al.*, 2007) High-dose systemic injections of IFN- α can suppress RRP regrowth post-operatively, but at the price of inducing fevers and other significant side effects. Longterm delivery is not well tolerated. Some patients do experience successful remission of their papillomas, but most have a rapid rebound after termination of IFN treatments. (Arney *et al.*, 2010)

Hydroxy 2 phosphonmethoxy propyl cytosine (HPMPC), (Cidofovir / Vistide) and other acyclic nucleoside analogs have been used off-label and compassionately in RRP patients. The prodrugs are delivered to the tissue by intra-lesional injection, and they require phosphorylation once inside the cells to become a tri-phosphate equivalent, the immediate substrate for DNA or RNA polymerases. It is not known which kinase is responsible for their conversion to the triphosphate equivalent. On a precautionary note, cidofovir is not a chain terminator after incorporation (in contrast to most other nucleotide analogs used to inhibit DNA or RNA polymerases). (Munoz *et al.*, 2006) Rather, it has a 3' ϕ hydroxyl group, it can be incorporated within growing polynucleotides). Its ultimate mechanism of inhibition is that polymerases slow down when encountering acyclic nucleoside phosphonates, as the normal steric orientation of the sugar ring is absent. Incorporated HPMPC apparently causes RNA and DNA polymerases to pause or stop, of particular concern is that the incorporated nucleotide analog can be mutagenic because of poor templating. The long-term consequences of cidofovir in people treated for RRP remain unknown, and considerable caution in its use should be exercised. Some patients respond well to cidofovir and their lesions are minimized over time, but many others have little or no benefit (Leuven, 2008) and may even experience vocal cord 'stiffness', perhaps because healing is delayed or prevented.

Recently, several new agents have entered clinical trials (Cox II inhibitors), have been advanced by RRP patients (artemisinin), or are in use by some ENT surgeons (e.g. intralesional injections with the mumps vaccine or Avastin). Cox II inhibitor (e.g. celecoxib / Celebrex) and other new agents. As discussed above, the enzyme COX-2 and its major product PGE2 are expressed in HPV-induced respiratory papillomas and promote the growth and survival of the cells. A pilot clinical trial of celecoxib therapy for RRP showed striking responses, with 3 / 3 patients free of disease (A. Abramson, *et al.*, 2010).

A large multicentered double-blinded placebo-controlled study of celecoxib therapy has been funded by the National Institutes of Health and is currently underway. Included in the design of the trial are a number of studies to address the mechanism of celecoxib efficacy in vivo. Artemisinin, artesunate, and related compounds are antimalarial agents derived from wormwood. They are cytotoxic against HPV immortalized cells or cervical carcinoma cells, but not against normal cervical cells (Reisinger *et al.*, 2008). Delivered orally, they have shown anecdotal benefits against laryngeal papillomatosis. The mechanism of action may involve selective generation of free radicals and oxidative stress in active papillomas. Additional research and validation are essential. (Schiffman *et al.*, 2003).

Mumps vaccine (or the MMR vaccine) injection directly into papillomas can result in remarkable regression of recalcitrant papillomas in some patients (Schwarz *et al.*, 2009). A presumed mechanism of action is local boosted immune response (because essentially all RRP patients received mumps or MMR vaccination as infants) with collateral suppression of HPV activity.

Bevacizumab (Avastin) is an anti-angiogenic agent that, when injected sublesionally following angiolytic KTP laser treatment, can prevent rapid regrowth of papillomas of the larynx and nearby upper aerodigestive tract sites (Wheeler, 2009). In summary, adjuvant treatments of RRP have involved a great deal of trial and error.

The described approaches benefit some but definitely not all patients. They remain rather non-specific and appear to be limited by idiosyncrasies associated with individual patients for reasons not understood. Proscriptions and contraindications – Pharmaceutical compounds such as acyclovir and gancyclovir have shown efficacy in treating infections caused by the herpesviruses because these large viruses encode a unique thymidine kinase which is capable of turning these analogs into substrates for nucleic acid synthesis. However,

HPVs strictly rely on host enzymes to supply the substrates and hence these agents would not have any specificity against HPV lesions. Other treatments (5-fluorouracil) that have been tried clinically should be avoided as they are likely to block cellular DNA synthesis or introduce unwanted mutations in cellular genes. In no case should ionizing radiation be used to treat any benign papilloma lesion, as the resulting DNA damage and strand breaks could promote chromosomal DNA instability and viral DNA integration and accelerate neoplastic progression. Such unintended consequences did result from attempts to treat laryngeal papillomas with radiation in the early days of clinical radiology.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out in two hospitals in Lokoja metropolis of Kogi state. Lokoja is located at latitude 7.8 degree north and 6.7 degree and Longitude 06 44'E and 07 48'E. Lokoja lies at the confluence of the Niger and the Benue rivers and is the capital of Kogi state. Lokoja shares boundaries with Okene to the North East; Ajaokuta to the East; Kabba to the south; Yagba to the West and Koton-Karfe to the north. Lokoja is also a Local Government in Kogi State with the headquarters in Koton- Karfe.

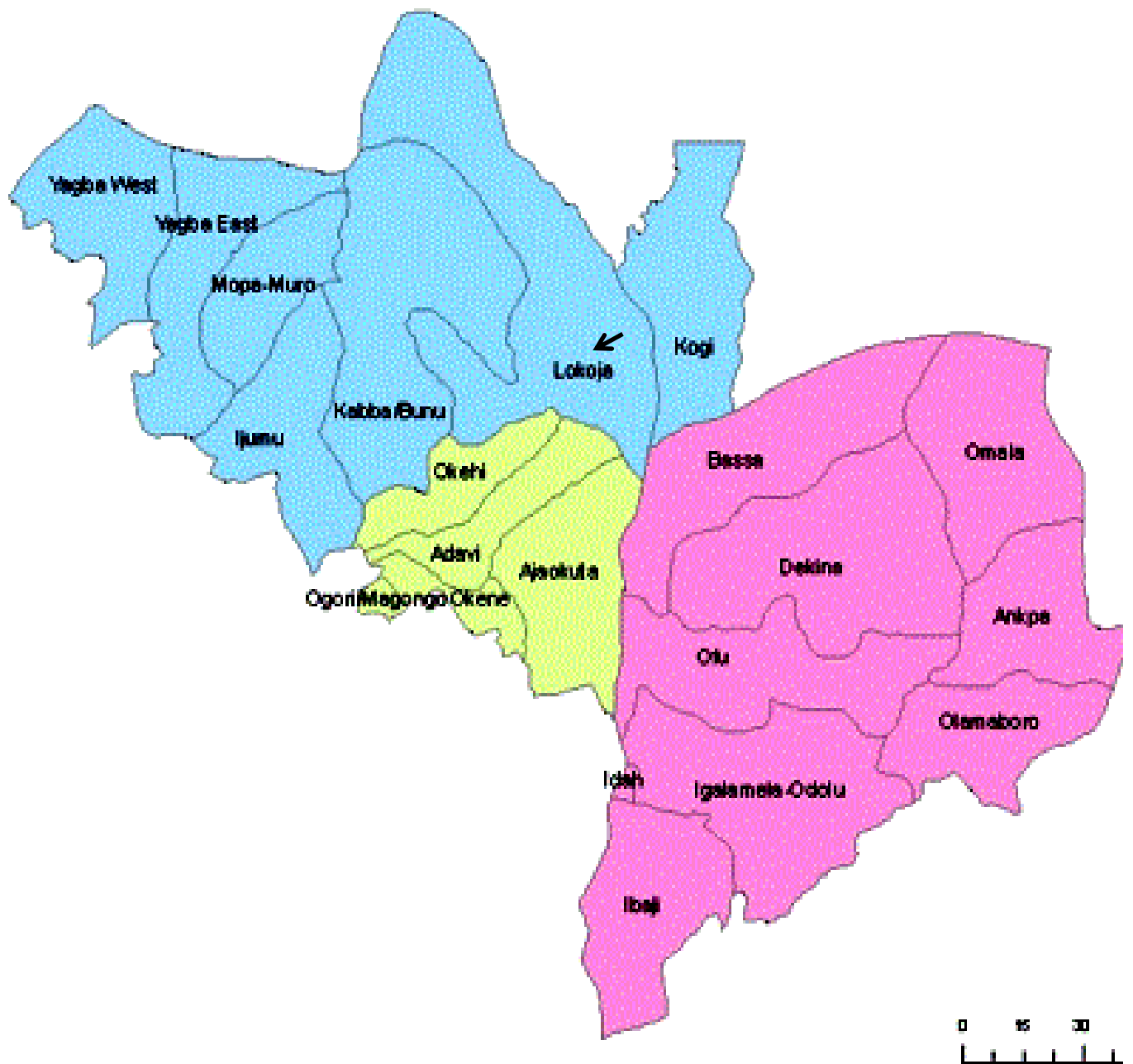


Figure 3.1 Map of Kogi State with an Arrow Showing the Study Area

3.2 Study Population

Study population comprised of 380 pregnant women of all ages and 20 non pregnant women making a total of 400 women from all works of life attending antenatal clinic in both hospitals, (Kogi State Specialist hospital and Federal Medical Centre, which are both located in Ilokoja) Who are willing and have consented to be enlisted in the study. For those below the ages of 18, consent was sought from their family members.

3.3 Control Population

Female patients visiting the hospital other than pregnant women were used as control population.

3.4 Inclusion Criteria

This research study included pregnant women of all ages attending antenatal clinic within the study period that expressed interest in participating and gave consent to the interview irrespective of the trimester or stage of their pregnancy.

3.5 Exclusion Criteria

All pregnant women who declined to participate in the study or failed to give consent to the interviewer and those below the age of 18 years whose family members did not consent for their enrolment in this study.

3.6 Ethical Consent

Ethical approval (Appendix II) was obtained from the Research and Ethical Committee of Kogi State Ministry of Health, Kogi State and Ethical committee of the Federal Medical Center, Ilokoja in order to be allowed to carry out the research. Consent form (Appendix II) was issued to individuals whose blood were to be collected to indicate that they were willing and have voluntarily agreed to participate in this research without any compulsion.

3.7 Sample Size Determination

The sample size for this study was determined by using the formula by Naing *et al.* (2006) as below and a prevalence of 42.9% from a previous study (Aminu *et al.*, 2004)

$$N = z^2 pq/d^2$$

Where, N = Sample size

Z= Standard normal distribution at 95% limit=1.96;

P= Prevalence rate from previous study=42.9% (Aminu *et al.*, 2014).

Q=1-P;

d=absolute desire precision (allowable error) = 5% or 0.05

Thus, N= 376.4

The calculated sample size was 376, hence a total of 400 samples were collected from Federal Medical Center and Kogi State Specialist Hospital Ilokoja. Out of the 400 samples collected 380 were from pregnant women (study population) and 20 were from non-pregnant women (control population). A total of 230 blood samples were collected from Federal Medical Center and 170 blood samples were also collected from the Kogi State Specialist Hospital with (10 non-pregnant women from each hospital) as the control population)

3.8 Data Collection

Prior to the sample collection a semi-structured questionnaire was administered which included occupation, marital status, questions on socio-demographic (e.g., age, parity) clinical information and risk factors (e.g., family history of cancer, contraceptive use, smoking, other transmissible

infections age at first sexual intercourse immune/nutritional patterns of the participants were obtained).

3.9 Collection of Samples

The samples were collected with the help of laboratory technicians, between the months of June and October 2015. Using a sterile disposable syringe, 10ml of venous blood was collected aseptically by a clinician in divided portions of 5ml from each patient using 5ml plain vacutainer tubes with their needles. The tubes were then labeled appropriately with patient's laboratory number. The sample portions meant for haematological analysis were then dispensed into EDTA bottles.

Sera from the blood sample for ELISA technique were separated by allowing to clot at room temperature, followed by centrifugation at 250rpm for 5 minutes. The sera were then removed using clean Pasteur pipettes, transferred into serum containers and stored at -20degrees Celsius in the refrigerator, until when required.

3.10 Laboratory Diagnosis using Enzyme-Linked Immunosorbent Assay

Serum samples were analyzed using Enzyme-Linked Immunosorbent Assay (ELISA) for HPV IgG Using the diagnostic automation, (ELISA HPV 16 IgG kit).The haematological parameters including packed cell volume (PCV), erythrocyte sedimentation rate (ESR) and Whole blood count (WBC) or Differential count were also carried out.

3.10.1 Principle of the Assay

The HPV IgG antibody test kit is based on the principle for the enzyme immunoassay (EIA) micro titer wells are coated with HPV antigens. This constitutes the solid phase. Diluted serum is added to the wells, any antibodies specific for the antigens will bind to the solid phase. After incubation for 60 minutes, at 30⁰C, the plate is rinsed with wash solution to remove unbounded

material. Anti-human IgG conjugated to an enzyme is allowed to react with the immune complex. The excess conjugate is removed by washing after which an appropriate substrate is added with which the conjugated enzymes reacts producing a colored derivative. The color development is terminated by the addition of a stop solution. The color intensity is proportional to the level of specific antibody bound and can be quantified spectrophotometrically at wavelength 450nm with a selected reference.

3.10.2 Test Procedure

All samples and reagents were brought to room temperature on bench and gently mixed. The washing buffer was prepared by adding 10mls of distilled water to 10x wash concentrate, shaken to mix well, and then made up to 1 liter with distilled water.

A 96 well plate microtiter plate was labeled and positive and negative calibrators and samples were labeled according to labels of the wells of the micro titer plates. A 1:40 dilution of samples, control positive and negative, cut off calibrator, negative and positive calibrators were prepared by pipetting 195ul of sample diluent using 200ul multiple channeled micropipettes into each labeled well. About 5ul of the test samples, negative and positive controls, cutoff and calibrators were pipetted using 10ul micro pipette into the 195ul of sample diluents into the corresponding wells. The micro titer plate was then shaken gently on the bench to mix the contents.

A 96 well micro titer plate coated with HPV antigens in each well, was labeled the same way the plane micro titer plane micro titer plate was labeled, and 100ul of diluted sera samples calibrators cutoff calibrator and positive and negative controls were pipetted using 200ul multiple channeled micropipette and dispensed into corresponding labeled wells of the HPV antigens coated micro-titer plates. 100ul of sample diluents was dispensed into 1A well position as blank. The holder was tapped to remove air bubbles from the liquid and mixed well then incubated for 30minutes at room temperature.

The diluted samples, positive and negative controls, cut off; calibrator and blank were removed from wells and washed 3 times with washing buffer then blotted with tissue paper

A 100ul of enzyme conjugates was pipetted using multiple channeled micro pipettes and dispensed into each wells and incubated for 30 minutes at room temperature after which the enzyme conjugates was removed from all the wells and washed three times with wash buffer as before and followed by blotting with tissue paper.

About 100ul of TMB chromogenic substrates was pipetted with multiple channeled micro pipettes and dispensed into each well the incubated for 30 minutes at room temperature

Finally 100ul of 2N HCl was added with the use of multiple channeled micro pipettes to each well to stop the reaction, making sure that they were no air bubbles before reading. The optical density (OD) was then read with GF-3000 micro plate reader (B brand scientific instrument England) at 450nm wavelength.

3.11 Determination of Haematological Parameters

The rationale behind conducting these tests was to ascertain if there was direct association between the presence of HPV16 IgG antibodies and some hematological parameters and if a decrease or an increase in the normal ranges of these parameters are relatable to the infection. These include differential count (Erythrocytes, Lymphocytes, Neutrophils, Basophils), PCV (packed cell volume) were considered.

3.11.1 Determination of Packed Cell Volume, By Centrifugation Method

Procedure: The fresh blood sample was gently mixed in a heparinized capillary tube by capillary action; the blood was allowed to enter the tube stopping at 10-15mm from one end by capillary

action. The tube was then wiped and sealed at the dry end by heating in a fine Bunsen burner flame and then placed into one of the centrifuge plate slots, with the sealed end against the rubber gasket of the centrifuge plate and was spun at 10,000-15000rpm for 15 minutes. The record of the patient number was kept against centrifuge plate number and centrifuged for five times and the PCV was read in the micro hematocrit reader and the results expressed in percentage. The PCV was performed in duplicate.

3.11.2 White Blood Cell Count/Leukocyte Count

Principle: A sample of whole blood is mixed with a weak acid solution that Lyses non-nucleated red blood cells. Following adequate mixing, the specimen is introduced into a counting chamber where the white blood cells (leukocytes) in a diluted volume are counted.

Reagent: white-count diluting fluid. Either of the following diluting fluids will be used: two percent acetic acid will be added to 2ml glacial acetic acid to a 100ml volumetric flask. The mark will be diluted with distilled water or 1% hydrochloride acid will be prepared by adding 1ml hydrochloric acid to a 100ml volumetric flask. The mark will be diluted with distilled water.

Procedure: A well-mixed venous blood by capillary action was marked exactly to 0.5 marks in white blood cell diluting pipette. The blood column was ensured to be free of bubbles. Excess blood was wiped from the outside of the pipette to avoid transfers of cells to the diluting fluids and care was taken not to touch the tip of the pipette with the gauze.

The diluting fluid was drawn immediately to the 11 mark while rotating the pipette upright to prevent air bubbles in the bulb. The contents in the pipette were mixed for 3-5 minutes to ensure even distribution of cells. And the unmixed and relatively free cells from the capillary portion of the pipette were removed.

The fluid was held at an angle of 45° and the tip of the pipette to the cover glass was touched by

the counting chamber. The mixture was then allowed to flow under the cover glass until the counting chamber was completely charged; the opposite chamber of the hemacytometer was filled. The cells were allowed to settle for about three minutes and was observed under low power magnification and reduced light, the focus on the ruled area was observed for even distribution of cells. The white cells were counted in the four 1sq mm corner areas corresponding to those marked A, B, C and D in each of the two chambers. All the white cells lying within the square and those touching the upper and right-hand center lines were not counted. The white cells that touch the left-hand and bottom lines were counted.

3.11.3 White Blood Cell Differential Count

Procedure: A film of blood was prepared on the slide by putting a drop of blood from the EDTA bottle on the slide and using another slide rubbing horizontally against each other after which the slide was labeled with the patients name, the film was adequately air dried in the space of 3-5 minutes and was dip in the stain for 10 seconds (combination of polychrome methylene blue and eosin stain) and then dipped in distilled water for 20 seconds and observed under the microscope using the x100 objective lens after smearing oil over length of slides. The white blood cell count was noted by counting the number of white cells under the x100 lens objective. While viewing, the resultant cell appearances were noted and recorded.

3.12 Data Analysis

The data obtained from the questionnaire and the results of the laboratory analysis were entered into the Microsoft excel and analyzed using SPSS (statistical package for social sciences version 20). The results obtained were reduced to percentages and figures. The Pearson chi square test at 95% level of significance was used to determine the relationships between the demographic data and prevalence rates, and the Pearson chi square of independence was used to measure the level of association between the haematological parameters and the HPV 16

prevalence. p-value ≤ 0.05 was taken as significant.

CHAPTER FOUR

4.0

RESULTS

4.1 Analysis of the Study Population

Analysis of the respondents by age (Table 4.1) revealed that respondents in age group 26-30 years had the highest frequency (40.5%: 162/400) while respondents in age group 15-20 years constituted the least (1.8%: 7/400). Majority of the respondents were married (85%: 340/400) while the single women were 20 (5.0%). Majority of the respondents had a family history of cervical cancer (35%:140/400)while those without the history of cervical cancer were more (65%:240/400).The distribution of the respondents by their highest level of education revealed that majority of the respondents had obtained a secondary education (45%: 180/400) while 27.5% (110/400) had just primary education. Distribution of the respondents by their trimester of pregnancy showed that majority of the respondents were in their second trimester of the pregnancy (43.5%: 175/400) while those in their first trimester constituted the least population (12.5%:50/400).

Information concerning the sexual and reproductive characteristics of the respondents was obtained, as these features predispose to HPV infection. Considering the marriage type of the respondents (Table 4.2), majority of the respondents were monogamous (65%: 260/400) while 35% (140/400) practiced polygamy. A good number of the respondents (38.3%: 153/400) had their first sexual contact at ages 21 and above, while 10% (45/400) had sex at age 31 and above. Most of the respondents had single sexual partners (91.6%: 365/400) while 8.8% (35/400) had a history of multiple sexual partners. Classifying the respondents base on parity (number of times a woman has given birth) revealed that most of them (36.3%: 145/400) were nulliparous (have never given birth before), 31.5% (126/400) were multiparous (have given birth to 2-3 times), 27.8% (111/400) were primiparous (have had only 1 child) and 4.5% (18/400) were grand

multiparous (have had five or more children

Table 4.1: Distribution of Respondents by some Socio-demographic Features.

Parameter	Number Screened	Percentage (%)
Age (years)		
15-20	7	1.8
21-25	80	20.0
26-30	162	40.5
31-35	74	18.5
36-40	67	16.7
41-45	10	2.5
Marital Status		
Single	20	5
Married	340	85
Divorced	30	7.5
Widowed	10	2.5
History of Cervical Cancer		
Yes	140	35
No	260	65
Highest Level of Education		
Primary	110	27.5
Secondary	180	45
Tertiary	90	22.5
None	20	5
Gestation Period		
First Trimester	50	12.5
Second Trimester	175	45
Third Trimester	155	42.5
Not Applicable	20	

Table 4.2: Distribution of Respondents by Sexual and Reproductive Characteristics

Feature	Number Screened	Percentage (%)
Multiple Sexual Partners		
Yes	93	23.3
No	307	76.8
Age at Sexual Debut (Years)		
15-20	112	25.5
21-25	153	38.3
26-30	90	22.5
≥31	45	11.3
Parity		
Nulliparous	145	36.3
Primiparous	111	27.8
Multiparous	126	31.5
Grand Multiparous	18	4.5

4.2 Enzyme-Linked Immunosorbent Assay Results

From the results obtained using blood samples from 400 women, 40 (10%) were positive, with most cases (11.3%: 26/230) coming from Federal Medical Centre while (8.2%: 14/170) were from Kogi State specialist Hospital (Table 4.3).

Relating the presence of the IgG antibodies with the ages of the respondents revealed that respondents that were in age group 26-30 years had the highest prevalence of IgG antibodies to HPV type 16 (9.3%: 15/162) while those in age group 15-20 years had the lowest prevalence (0.0%: 0/7) (Table 4.4). Age therefore, was not statistically associated with the presence of IgG antibodies to HPV type 16 in the study population ($\chi^2 = 8.805$, $df = 5$, $p=0.117$). Distribution of HPV infection based on marital status revealed that the married women had the IgG antibodies to HPV type 16 prevalence of 8.5% (29/340) while the widows had a prevalence of 30% (3/10). There was no statistically significant association between marital status and IgG antibodies to HPV type 16 ($\chi^2 = 7.298$, $df=3$, $p = 0.063$).

Taking level of education as a factor, it was observed that women that had no form of education had the highest rate of infection (25%: 5/20) while those that attended tertiary institutions had the lowest (6.7%: 12/180). There was a statistically significant association between level of education and IgG antibodies to HPV type 16 ($\chi^2 = 8.962$, $df = 3$, $p = 0.030$). Women that fell into the “Others” category had the highest rate of HPV infection, (25.0%:7/28) while the civil servants had the lowest (8.4%: 9/250). There was a statistically significant association between occupation and IgG antibodies to HPV ($\chi^2 = 9.222$, $df = 3$, $p = 0.026$)

The sexual and reproductive features of the respondents were analyzed as shown in Table 4.5. It was observed clearly that although most of the respondents were monogamous, HPV type 16 IgG antibodies was however, highest in the few that practiced other types of marriage (15%: 3/20) followed by those that practiced polygamy (14%: 15/107) ($\chi^2 = 3.619$, $df = 2$, $p = 0.164$).

The respondents that had their first sexual contact at the ages of 15-20 had the highest rate of IgG antibodies to HPV type 16 (15.9%: 17/112), while those that had their first sex at between ages 31 and above had the lowest 4.4% (2/45) ($\chi^2 = 5.671$, $df = 3$, $p = 0.129$).

In relating the presence IgG antibodies to HPV type 16 with multiple sex partners, it was observed that majority of the women said they only had one sex partner and had the prevalence of 10.4% (32/307) and a prevalence of 8.6% (8/93) for those with multiple sex partners. ($\chi^2 = 0.263$, $df = 1$, $p = 0.068$)

Relating parity with IgG antibodies to HPV type 16 showed that women that had five or more deliveries (Grand multiparous) had the highest prevalence rate of 22.2% (4/8), while nulliparous women had the lowest rate (5.5%: 8/145). Parity was not statistically associated with the presence IgG antibodies to HPV type 16 in the study but we observed an increasing infection with increase in number of deliveries ($\chi^2 = 7.326$, $df = 3$, $p = 0.062$).

Table 4.3: Seroprevalence of Human Papilloma Virus Type 16 IgG Antibodies among women Attending Clinics in Lokoja, Kogi State, Nigeria.

Hospital	Number Analysed	Number Positive (%)	P Value
FMC	230	26 (11.3)	0.312 ^{ns}
KSSH	170	14 (8.2)	
TOTAL	400	40 (10)	

KEY

FMC=Federal Medical Center.

KSSH= Kogi State Specialist Hospital

Table 4.4: Seroprevalence of Human Papilloma Virus 16 IgG Antibodies based on some Socio-demographic Features among Women Attending Clinics in Lokoja, Kogi State, Nigeria.

Parameter	Number Screened	Number Positive (%)	P Value
Age (years)			
15-20	7	0 (0.0)	0.117 ^{ns}
21-25	80	4 (5.0)	
26-30	162	15 (9.3)	
31-35	74	9 (12.2)	
36-40	67	9 (13.4)	
41-45	10	3 (30.0)	
Marital Status			
Single	20	3 (15.0)	0.063 ^{ns}
Married	340	29 (8.5)	
Divorced	30	5 (16.7)	
Widowed	10	3 (30.0)	
Highest Level of Education			
Primary	110	15 (13.6)	0.030*
Secondary	90	8 (8.7)	
Tertiary	180	12 (6.7.)	
None	20	5 (25.0)	
Occupation			
Civil Servant	250	21 (8.4)	0.026*
Business	112	12 (10.7)	
Farming	60	10 (16.7)	
Others	28	7 (25.0)	

Table 4.5: Seroprevalence of Human Papilloma Virus Type 16 IgG Antibodies based on some Sexual and Reproductive Features among Women Attending Clinics in Lokoja, Kogi State, Nigeria.

Parameter	Number Screened	Number Positive (%)	P Value
Type of Marriage			
Monogamy	273	22 (8.1)	0.164 ^{ns}
Polygamy	107	15 (14.0)	
Not Applicable	20	3 (15.0)	
Multiple Sexual Partners			
Yes	93	8 (8.6)	0.608 ^{ns}
No	307	32 (10.4)	
Age at Sexual Debut			
15-20	112	17 (15.9)	0.129 ^{ns}
21-25	153	12 (7.8)	
26-30	90	9 (10.0)	
≥31	45	2 (4.4)	
Parity			
Nulliparous	146	8 (5.5)	0.062 ^{ns}
Primiparous	116	12 (10.8)	
Multiparous	100	16 (16.0)	
Grand Multiparous	18	4 (22.2)	

The prevalence of IgG antibodies to HPV type 16 according to pregnancy status revealed that women that were in their first trimester of pregnancy had the highest prevalence 16% (8/50) of IgG antibodies to HPV type 16. The infection was observed more in the pregnant respondents (10.3%: 39/380) with no statistically significant difference ($\chi^2 = 0.585$, $df = 1$ $p = 0.444$). (Table 4.6)

Some risk factors that predispose to IgG antibodies to HPV type 16 were examined as shown in Table (4.7). The result revealed that IgG antibodies to HPV type 16 was most prevalent (24.2%: 8/33) in women that have never used oral contraceptives while it was lowest (5.7%: 8/141) in those that used contraceptive for 5-9 years. There was a strong statistical association between IgG antibodies to HPV type 16 and the use of oral contraceptives ($\chi^2 = 15.115$, $df = 3$, $p = 0.002$).

The prevalence according to smoking showed that there was no statistical association between IgG antibodies to HPV type 16 and smoking ($\chi^2 = 0.046$, $df = 1$, $p = 0.830$.) it was only 2 out of the 23 respondents that smoked that had the infection.

Analysis of the co-infection of IgG antibodies to HPV type 16 and other sexually-transmitted organisms revealed that women that had other Sexually-Transmitted Infections (STI's) had a higher prevalence rate of IgG antibodies to HPV type 16 (12.1%: 24/198) compared to women that did not have other STI's (7.9%: 16/202). There was no statistically significant association between infection with other sexually-transmitted organisms and IgG antibodies to HPV type 16 ($\chi^2 = 1.960$, $df = 1$, $p = 0.161$) (Table 4.8)

The prevalence according to family history of cancer showed that respondents that had a family history of cancer had a prevalence of 38.9% (7/18) as opposed to those with no family history of cancer with a prevalence of (8.6%: 33/382). There was a statistical association between IgG antibodies to HPV type 16 and family history of cancer. ($\chi^2 = 17.478$, $df = 1$, $p = 0.000$).

Table 4.6 Seroprevalence of Human Papilloma Virus 16 IgG Antibodies based on Age of Pregnancy among women Attending Clinics in Lokoja, Kogi State, Nigeria.

Age of Pregnancy	Number Analysed	Number Positive (%)	P Value
First Trimester	50	8 (16.0)	0.350 ^{ns}
Second Trimester	150	12 (8.0)	
Third Trimester	180	19 (10.6)	
Not Pregnant	20	1(5.0)	

Table 4.7: Seroprevalence of Human Papilloma Virus 16 IgG Antibodies based on Pregnancy Status of women Attending Antenatal Clinics in Lokoja, Kogi State, Nigeria.

Pregnancy	Number Analysed	Number Positive (%)	P Value
Yes	380	39 (10.3)	0.444 ^{ns}
No	20	1 (5.0)	
Total	400	40 (10)	

Table 4.8: Seroprevalence of Human Papilloma Virus 16 IgG Antibodies based on some Risk Factors among Women Attending Clinics in Lokoja, Kogi State, Nigeria.

Parameter	Number Screened	Number Positive (%)	P Value
Years of Oral Contraceptives use			
1-4	105	16 (15.2)	0.002*
5-9	141	8 (5.7)	
≥10	121	8 (6.6)	
Never used	33	8 (24.2)	
Smoking			
Yes	23	2 (8.7)	0.830 ^{ns}
No	377	38 (10.1)	
Presence of other STI's			
Yes	198	24 (12.1)	0.161 ^{ns}
No	202	16 (7.9)	
History of Cancer			
Yes	18	7 (38.9)	0.000*
No	382	33 (8.6)	

4.3 Haematology Results

Hematological pattern due to IgG antibodies to HPV type 16 among pregnant women in Kogi State was ascertained using blood samples of 400 women analyzed using a hemocytometer (machine) this result is shown in table (4.10). From the result of the overall PCV conducted for all the respondents it was found that 1% of them (4/400) had high PCV values, 7.5% (30/400) of them had results in the normal range and 91.5% (366/400) of the respondents had low PCV results. There was no statistically significant association between HPV 16 antibodies and PVC. ($\chi^2 = 1.448$, $df = 2$, $p = 0.485$). (Table 4.10)

Results for the white blood cell count (WBC) showed that 70.5% (282/400) of the respondents had high counts, 28.7% (115/400) had normal counts, while 0.7% (3/400) of the respondent had low counts. The results showed that there was a statistically significant association between white blood cell (WBC) and the HPV 16 IgG antibodies ($\chi^2 = 15.115$, $df = 2$, $p = 0.004$). (Table 4.10)

Similarly from the results of the differential blood counts conducted 10.4% (41/400) of the respondents had high lymphocyte counts 88.5% (354/400) of the respondent had normal results while 1.2% (5/400) had low lymphocyte counts. The result showed that there was a statistically significant association or relationship between lymphocyte count and the prevalence ($\chi^2 = 348.838$, $df = 2$, $p = 0.000$). (Table 4.10)

From the results we also found that, 46.3% (185/400) of the respondents had high monocyte counts 53.2 % (213/400) had normal counts while 0.5 % (2/400) had low monocyte counts. The results showed there was a statistically significant association between monocyte count and the prevalence of papilloma virus 16 IgG antibodies ($\chi^2 = 30.433$, $df = 2$, $p = 0.000$). (Table 4.10)

Also, the results showed that 8.7% (35/400) of the respondents had high neutrophil counts,

88.7% (355/400) of them had had normal results while 2.5% (10/400) of the respondents had low counts. The result showed that there was a statistically significant association or relationship between neutrophil count and the prevalence of human papillomavirus type 16 IgG antibodies ($\chi^2 = 306.497$, $df = 2$, $p = 0.000$). (Table 4.10)

It was found from the results that 4.8% (19/400) of the respondents had high basophil counts, 92.7% (371/400) had normal counts and 2.5% (10/400) had low counts. This showed that there was no statistically significant association or relationship between basophil count and the prevalence of HPV16 IgG antibodies. ($\chi^2 = 0.006$, $df = 2$, $p = 0.0997$). (Table 4.10)

The results also showed that 7.8% (31/400) of the respondents had high eosinophil counts 83.7% (331/400) had normal counts while 8.5% (34/400) had low counts. The result showed that there was no significant statistical association or relationship between Eosinophil count and the prevalence of papilloma virus type 16 IgG antibodies. ($\chi^2 = 0.561$, $df = 2$, $p = 0.756$) (Table 4.10)

Table 4.9: The Pattern of Haematological Parameters among the Study Population.

Variable		Number of samples	Prevalence (%)
Packed Cell Volume			
High	4	1	
Normal		30	7.5
Low		366	91.5
White Blood Cell Count			
High	282	70.5	
Normal	115	28.7	
Low	3	0.7	
Lymphocyte Count			
High		41	10.4
Normal	354	88.5	
Low	5		1.2
Monocyte Count			
High	185	46.3	
Normal	213	53.2	
Low		2	0.5
Neutrophil Count			
High	35	8.7	
Normal	355	88.7	
Low	10	2.5	
Basophil count			
High	19	4.8	
Normal	371	92.7	
Low	10	2.5	
Eosinophil Count			
High	69	17.3	
Normal		331	82.8
Low		0	0

Key

PCV Normal Range 35-47%, White Blood Cell Normal Range 4.5-11.0X 10⁹b, Lymphocyte Count 0.0-4.0%, Monocyte Count Normal Range 4.7-12.5%, Neutrophil Count Normal Range 35-80%, Basophil count Normal Range 2-4%, Eosinophil Count Normal Range 0.0-6.0%.

4.4 Relating the Pattern of Haematological Parameters with the HPV16 IgG Antibodies Positive and Negative Results

From the hematological assays performed using the blood samples collected from the 400 respondents, out of the 40 that tested positive to HPV16 antibodies, 2.5% respondents had high counts and the woman with this high PCV is a non-pregnant woman from the control group used in this study. 10 % of those who tested positive had normal results and 87.5% of the women had low PCV results. Out of the 360 respondents that tested negative to the infection, 8.6 % of them had high counts, 7.2% had normal range results while 91.9% of the respondents had low counts, of those women that had really low PCV majority of them were pregnant women in their second and third trimesters respectively. (Table 4.10)

The results of the white blood cell (WBC) count showed that, among those that had the infection, 80% (32/40) of them had high WBC count, and 17.5% of them had normal counts while 2.5% (1/40) of them had low counts. Among those without the infection, 64.9% of them had high WBC counts, while 27.7% (108/360) had normal counts and 0.5 % had low counts. (Table 4.10)

From the results of the differential count, out of the 40 respondents that tested positive to the antibodies, 95 % of them had high lymphocyte count, 2.5 % of them had normal counts and 2.5 % also had low counts. Among those without the infection, 0.8 % of them had high lymphocyte count, 98.1% of them had normal counts while 1.1 % of them had low lymphocyte counts. (Table 4.10)

It was also found from the result of the differential count that, out of the 40 that tested positive to the infection, 82.5 % had high neutrophil counts, 12.5 % of them had normal counts while 5 % of them had low neutrophil counts. From the HPV negative group, 0.6 % of them had high neutrophil counts, 97.2 % of them had normal counts while 2.2 % of them had low counts. (Table 4.10)

The results also showed that, from the 40 respondents who had the infection, 87.5% of them had high Monocyte counts, 12.5% of them had normal counts, while 0% of them had low counts and among those that were negative, 41.6% of them had high monocyte counts, 57.8% had normal counts while 0.5% had low counts.

Similarly, from the results, we found that out of the 40 that tested positive to the infection, we found that 5 % (2/40) of them had high basophil counts, 92.3% (37/40) of them had normal counts and 2.5 % (1/40) of them had low basophil counts. Out of those that had no infection, 4.7% (17/360) of them had high counts, 92% (334/360) had normal counts and 2.5 % (9/360) had low basophil counts.

Finally, from the results, we found that of the 40 that tested positive to HPV 16 IgG antibodies, 5% (2/40) of them had high eosinophil counts, 87.5%(35/40) had normal counts while 7.5%(3/40) had low eosinophil counts. while among those without the infection, 8 % (29/360) had high eosinophil count, 83.3 % (300/360) had normal counts, and 8.6% (31/360) had low counts.

Table 4.10: The Pattern of Haematological Results for both the Positive and the Negative Samples

Variable	No +ve	Prevalence (%)	No -ve	Prevalence (%)	Pvalue
Parked Cell Volume					
High	1	2.5	3	8.6	
Normal	4	10	26	7.2	0.485
Low	35	87.5	331	91.9	
White Blood Cell Count					
High	32	80	250	64.9	
Normal	17	17.5	108	27.7	0.004
Low	1	2.5	2	0.5	
Lymphocyte Count					
High	38	95	3	0.8	
Normal	1	2.5	353	98.1	0.000
Low			1	2.5	4 1.1
Monocyte Count					
High	35	87.5	150	41.6	
Normal	5	12.5	208	57.8	0.000
Low		0	0	2 0.5	
Neutrophil Count					
High			33	82.5	2 0.6
Normal	5	12.5	350	97.2	0.000
Low			2	5	8 2.2
Basophil Count					
High			2	5	17 4.7
Normal			37	92.3	334 9.2 0.099
Low			1	2.5	9 2.5
Eosinophil Count					
High	5	12.5	60	16.7	
Normal		35	87.5	300	82.2 0.756
Low	0	0	0	0.0	

CHAPTER FIVE

5.0

DISCUSSION

Human Papilloma virus type 16 antibodies were detected in serum samples from 40 out of the 400 women studied, giving a seroprevalence of 10%. This indicates that the women had been infected and the virus is circulating among some women of the studied. This finding is important because HPV type 16 (HPV16) is the most common HPV type detected in tumors, accounting for 50% of cancers and their precursors, called high-grade squamous intraepithelial lesions (HSILs) (Bosch *et al.*, 1995). Preliminary studies have suggested that variants of HPV16 may show varying degrees of association with cervical neoplasia (Hildersheim *et al.*, 2001; Touze *et al.*, 2001). This result is comparable to the report of Okolo *et al.* (2010) and much higher than the 4.0% and 1.2% reported in reported in Ibadan and Enugu respectively (Ngokere *et al.*, 1996; Okesola and Fawole, 2001). The prevalence is slightly lower than 10.3% reported in Imo state (Ojiyi *et al.*, 2013). The prevalence is much lower than, 32% and 48% reported in, Tanzania and Brazil respectively (Termculen *et al.*, 1992; Armbruster *et al.*, 2000). The differences in the prevalence reported in the previous studies and the present study could be attributed to the fact that only HPV type 16 was considered in this study. This disparity in prevalence may also be explained by variations among the different study populations with varying exposures to different risk factors based on diverse socio-cultural and geographical differences. The Federal medical center Lokoja being the only tertiary medical facility and patients are referred there from hospitals in Lokoja and its environs with Kogi state specialist hospital, a second public hospital and 10% of the women attending antenatal clinic in both hospitals have been infected with HPV type 16, this percentage represents the actual seroprevalence of HPV type 16 in Lokoja.

Human Papilloma virus 16 IgG antibodies were found to be highest in participants that were between ages 41-45 years. The high HPV prevalence among older women may be explained by

HPV persistence and/or new incident infections due to changing sexual behavior and age-related changes of mucosal biology and immune competence (Leyh-Bannurah *et al.*, 2014; Assoumou *et al.*, 2016). The findings agree with those of Nuacler *et al.* (2007) and Ziziphor *et al.* (2010) who reported a higher HPV prevalence among older women but differ from the findings of Christine and Holschneider (2003), Pietro *et al.* (2008) and Ojiyi *et al.* (2013) that reported a higher prevalence among younger women. There was no statistical association between age and HPV prevalence and this agrees with the findings of Ojiyi *et al.* (2012).

Distribution of HPV IgG antibodies based on marital status revealed that those respondents that were widowed had the highest prevalence even though there was no significant association between marital status and HPV type 16 antibodies. This observation is similar to the findings of Schlect *et al.* (2001), Menacndez *et al.* (2010) and Mesmoudi *et al.* (2011) that reported a higher HPV prevalence among married women, because even though they are no longer with their husbands, they were once married. The finding however contrasts that of Tabora *et al.* (2015) that reported higher prevalence in single women. This finding also, does not agree with that of Aminu *et al.* (2014) that reported a similar HPV prevalence in both the single and married women. The absence of HPV infection in the single women could be because most of them take protective measures (using barriers) when having sex with their partners due to their fear of getting pregnant or contracting sexually-transmitted diseases. In addition, the number of single women enrolled in the study was small.

Women with lower levels of education were found to have a higher risk of HPV infection as shown in this study and another Nigerian study (Akarolo-Anthony *et al.*, 2013). Higher educational level among women is generally associated with increased knowledge/attitude towards HPV and its preventive measures with minimal risk factors for the infection (Chang *et al.*, 2013). This agrees with the findings of Ferrera *et al.* (2000) that more education plays in

fostering a lifestyle that reduce the risk of invasive cervical cancer. The data suggesting important elements of such a lifestyle include latter age at first sexual intercourse, a limited number of pregnancy, and greater likelihood of undergoing cytological screening and reduce exposure to carcinogen in household environments. This observation is similar to those of Marrazzo *et al.* (2001), Schlecht *et al.* (2001), Naucler *et al.* (2007) and Aminu *et al.* (2014) that reported highest prevalence in elementary educated women, there was a statistically significant association between HPV infection and education.

There was association between HPV type 16 antibodies and occupation. Among occupation groups, those with secured means of livelihood such as civil servants, business women and farmers were at least risk of HPV infection compared to others (students, apprentices, and petty traders) who do not have secured means of livelihood. The underlying factor might be multiple sexual partners as those who engage in sexual promiscuity do so for economic reasons (Okesola *et al.*, 2001). This is in line with the findings of Ojiyi *et al.* (2012) that reported a statistically significant association between occupation and acquisition of cervical HPV infection.

Although most of the respondents were monogamous, HPV infection however, was higher in the few that practiced polygamy, and marriage type was not associated with HPV infection. The higher prevalence of HPV type 16 antibodies in the polygamous women could be accounted for by the fact that Nigeria is a society where polygamy is generally accepted, a fraction of men and women (mainly men) may continue to have multiple sexual partners throughout their life and therefore re-infect themselves and their spouses (Fadahunsi *et al.*, 2013). This finding agrees with that of Aminu *et al.* (2014) that reported a higher prevalence amongst polygamous women.

Analysis according to number of sex partners revealed there was no statistically significant association between number of sexual partners and HPV infection. Underreporting of lifetime number of sexual partners, particularly among the most sexually active women, could contribute

to the lack of association between the occurrence of HPV antibodies and number of sexual partners (Vaccarella *et al.*, 2006). This is in contrast with the findings of Ojiyi *et al.* (2012) that observed multiple sexual partners and coital frequency to be the significantly associated with HPV infection and postulated that Women that had multiple sexual partners were 1.4 times more at risk of acquiring HPV infection than women with single sexual partners. This has also been observed for HPV positivity in other populations and in the relationship between sexual partners and cervical cancer risk.

There was no association between the occurrence of HPV type 16 antibodies and age at sexual debut. However, participant with age at sexual debut below 20 years have the highest rate of occurrence. This is because women who were exposed to sexual intercourse early have a greater tendency to having multiple sexual partners which predisposes them to HPV infection (Mosuro *et al.*, 2015). This finding is similar to the findings of Tabora *et al.* (2015), Naucner *et al.* (2007) and Smith *et al.* (2010) who found that increased risk was largely associated with women starting to have sex at a younger age.

In this study, there was no significant association between parity and HPV type 16 antibodies. However, it was seen that women that had five or more deliveries (Grand multiparous) had the highest infection rate of HPV compared to those that had fewer or no deliveries. This is because, Grand-Multiparity probably reflects early sexual exposure. Early age of first pregnancy, early marriage, early sexual exposure, marital instability, and subsequent multiple sexual partners through remarriages or otherwise are all closely related with synergistic effects. They all increase the chances of acquiring sexually transmitted diseases, including HPV (Ojiyi *et al.*, 2013). Other reasons to explain the preponderance of positivity among the grand multiparous are hormonal changes in pregnancy resulting in reducing immunity, to the exposure of the ectocervix during repeated child birth resulting in easy attachment of the human papilloma virus in addition to

damage of the cervical epithelium during childbirth and easy accessibility of the virus to be incorporated into the cellular matrix of the cervix (Sarma *et al.*, 2013; Nyengidiki *et al.*, 2016). This finding agrees with that Aminu *et al.* (2014) that reported no significant association between parity and HPV infection. This finding however, did not agree with the reports of Schiffman *et al.* (1996), Lorenzato *et al.* (2001), Trottier *et al.* (2008), Firnhaber *et al.* (2010) and Okolo *et al.* (2010) who found multiparous women at significant risk of acquiring genital HPV infection compared to their non-parous counterparts.

This study showed no association between the occurrence of human papilloma virus type 16 antibodies and trimester of pregnancy. However, the prevalence of HPV type 16 antibodies was higher during the first trimester and decreased as the pregnancy gets older. This may be due to clearing of the virus as pregnancy progresses (Nobbenhuis *et al.*, 2002). This finding is in consonance with the findings of (Nobbenhuis *et al.*, 2002). However, it differed from the findings by (Michelin *et al.*, 1997) who found higher prevalence as pregnancy progresses owing to possible selection of viruses due to hormonal and immunologic factors and decreased immunity during pregnancy.

The prevalence obtained among the pregnant (10.3%) and non-pregnant women (5.0%) used as control subjects shows a higher prevalence in the pregnant women compared to the non-pregnant women but there was no significant association between pregnancy and HPV infection. This non statistical association could be due to the small sample size of non-pregnant women compared to that of pregnant women. However the result is similar to a result of study of HPV among pregnant and non-pregnant women in Stockholm Sweden which showed no significant difference between prevalence in both groups (Pietro *et al.*, 2008). This is an important finding because the most feared consequences of HPV infection during pregnancy is the occurrence of juvenile laryngeal papillomatosis which is associated with a significant morbidity rate and linked

to mother- child transmission of the virus. (Silverberg and Luis, 2003)

The result of this study revealed that HPV type 16 IgG antibodies were most prevalent in women that have never used contraceptives and least in those that have used for > 10 years and above. This agrees with the findings of Marrazzo *et al.* (2001) and Naucler *et al.* (2007) that reported highest prevalence amongst women that have never used contraceptives. This finding disagrees with those of Pietro *et al.* (2008) and Ojiji *et al.* (2012) that reported a significant association between duration of contraception use and cervical HPV infection. This could be due to the difference in the contraceptive types (oral contraceptives and intrauterine devices) considered in both studies. Oral contraceptives when properly prescribed for individuals are safer than pregnancy and delivery does not need inserting and so do not tamper with the flora of the cervix. In contrast, IUDs when inserted can spread a genital infection into the uterus leading to pelvic inflammatory diseases and also may feast on mild medical conditions to bring about fatal outcomes. Human Papilloma Virus infection was found to be statistically associated with the use of oral contraceptives.

Analysis based on cigarette smoking showed no statistical association between the virus and smoking as a higher prevalence was observed in non-smokers than those who smoked; this contrasts the findings of Gillison *et al.* (2014) that tobacco exposure can increase a person's risk of being diagnosed with oral HPV type 16 and Mzarico *et al.* (2015) who found that smoking interferes in the increase of HPV infection prevalence and in an increased risk of cervical invasive neoplasia and cervical carcinoma and that risk also increases with more cigars smoked per day. The reason could be due to the fact that tobacco smoking was uncommon among the women studied, as only 23 of the participants admitted to have smoked out of which 2 had HPV antibodies.

The absence of any significant association between HPV and past sexually transmitted diseases might be accounted for by the fact that most women are very reluctant to disclose the details of previously sexually transmitted diseases or their treatment (Ojiyi *et al.*, 2013). However, history of sexual transmitted infections (STI) such as *Chlamydia trachomatis* and HIV (Castellsague *et al.*, 2007; Syrjanen *et al.*, 2007) have been identified to increase the risk of HPV. Indeed, some studies reported that the presence of *Chlamydia trachomatis* raises the acquisition and the persistence of HPV infection (Silins *et al.*, 2005; Lehtinen *et al.*, 2011) and seems to facilitate the penetration of HPV and the progress of cervical lesions by interfering in the immunological responses (Seraceni *et al.*, 2014). Women living with HIV are also at increased risk for HPV infection (De Vuyst *et al.*, 2012).

The distribution of HPV IgG antibodies among pregnant women by family medical history (example history of cervical cancer) shows that women who had a history of cancer in their family lineage had a highest prevalence than those who didn't, This agrees with the work of Radio *et al.*, (2013) that a family history of head and neck cancer is a marker for an increased risk of oral cavity cancer and should be taken into account to target prevention effort and screening.

Hematological analysis of the samples revealed a pattern of increasing count with the prevalence rates of the HPV 16 IgG antibodies in white blood cell counts. The result showed a significant association or relationship between lymphocyte count and the infection. This is because white blood cells protects the body against infection, if an infection develops, white blood cells attack and destroy the virus, bacteria or other organism causing it(CDC 2001).Similarly the result also revealed there was a significant association between the lymphocyte count and the HPV 16 IgG antibodies. This also agrees with literatures like (lab tests online. com) that because lymphocytes including (the T-cell lymphocytes, N K cell lymphocytes and the B-cell lymphocytes) which are

the three major kinds found in the human body are responsible for preventing the onset of infections in the body which may lead to many kinds of health implications, thus they play a vital role in helping the body fight against viral infections as well as the growth of tumors.

The results also revealed a significant association or relationship between the HPV 16 IgG antibodies and the result of the Monocyte count; this could be due to a number of reasons ranging from acute stress and auto immune diseases. Monocyte (macrophages) rid the body of old cells and debris and helps regulate the immune responses of the body, which is not farfetched because majority of the respondents were pregnant women and go through a lot of stress on a daily basis.

Furthermore, it was also found that there was a significant association or relationship between the HPV 16 IgG antibodies and the result of the neutrophil counts. those who had positive results had higher counts than those who didn't, this could be due to the fact that, neutrophils form the bulk of immune response, fast acting and swiftly arriving at the site of infection and act non-specifically, (destroy any invading microorganism) or could be as result of strenuous exercise and physiological factors such as pregnancy and labor. The finding does not agree with common knowledge and assertions of Luciano and Aldo, (2010) who found that HPV viremia and hematogenous dissemination does not occur and hence cellular immune response is unlikely. But in contrast to the above view, Bondaghi *et al.*, (2005) found that, since PBMCs (lymphocytes and Monocytes) migrate to sites of tissue inflammation and also to microorganisms from tissues or the blood stream we speculate that PBMCs execute the same functions for HPV infection as they do for many other viral infections Moreso, Pao *et al.*, (1991) found that HPV DNA could exist in polymorphonuclear cells of patients with genital HPV infection, and in the peripheral blood of cervical cancer patients (Kedzia *et al.*, 1992; Pao *et al.*, 1997; Tseng *et al.*, 1999) and in the sera or plasma of patients with cervical cancer (Liu *et*

al(2001; Dong et al 2002).

From the results of the data analyzed it was found that there was no significant association or relationship between the HPV16 IgG antibodies and the result of the basophil count this could also be as a result of pregnancy because a low basophil count can occur during pregnancy ovulation or stress. Similarly from the results obtained and the data analyzed there is no significant association between the HPV16 IgG antibodies and the result of the eosinophil count this could be due to the fact that, this immune cell is mainly linked to allergic diseases and infections from parasites asthma and other superficial infections like eczema and pimples.

Finally, from the results; there was no significant association between the results of the PCV and that of the HPV 16 IgG antibodies. This could be due to the fact that our study focus was on pregnant women and it is common knowledge that anaemia is found in most pregnancies.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

Human Papilloma Virus type 16 IgG antibodies were detected in 10% of the women enrolled in this study indicating that the women had been infected and the virus is circulating in Lokoja, Kogi State, Nigeria. Infection with HPV type 16 is a major factor contributing to the development of cervical intra epithelial neoplasia and invasive cervical carcinoma. Women aged 41-45 years and those who had their sexual debut aged 15-20 years had the highest seroprevalence of the antibody.

Infection with HPV type 16 varies with the women's reproductive characteristics, sexual behavior and socio demographic factors where there is a statistically significant association between the HPV 16 IgG antibodies and highest level of education and occupation.

Hematological analysis of the samples revealed a pattern of increasing count with disease in WBC, lymphocyte monocyte and neutrophil counts but not in PCV basophil and eosinophil counts, The high count was found to be predominant in the younger women having multiple sexual partners that never went beyond the secondary level of education. A change of pattern was observed in the PCV results where a slightly higher result of 8.6% was seen in the HPV negative group than the 2.5% recorded in those with the infection. Although further clarification is required, there have been promising results in the correlation between HPV and immune cells. However, the involvement of the infection in the change in these hematological parameters

remains inconclusive due to the modest nature of our sample size limiting the power of our study to detect significant associations of HPV with immune cells, nevertheless, our results highlight the need to further understand the correlation between HPV infection and some hematological parameters.

6.2 RECOMMENDATIONS

The prevalence of 10% for HPV 16 obtained in this study is very high, and as such, it is recommended that cervical cancer screening centers should be established in all areas of the state, and awareness campaigns as well as workshops should be organized, both at the federal, state and local government levels, that will serve to encourage young ladies and women to go for the screening exercises at least once in 3 years so as to prevent cervical cancer in future.

The risk factors that were found for HPV infections in this study include; early sexual initiation, multiple sexual partners, sexual enhancers and high parity, it is therefore recommended that the Government and other relevant bodies should make sufficiently available, vaccines for this HPV type in the study area, so that people, especially young girls be vaccinated before they become sexually active.

The involvement of the infection in the change in these hematological parameters remains inconclusive due to the modest nature of our sample size limiting the power of our study to detect significant associations of HPV with immune cells, therefore more studies are needed to further understand the correlation between HPV infection and some hematological parameters.

6.3 Limitations of the Study

The Limitations of this research were:

There was a problem of getting true information from the participants as many of them were not willing to disclose some personal information concerning their reproductive and sexual health as

they see revealing them as embarrassing which could have affected the data obtained.

There was no sufficient fund to have expanded this study as more immune cells should have been considered to see a more vivid picture and clear pattern with the presence or absence of the infection.

This study was done using only 1type-specific ELISA kit although it was considered because of the high prevalence and due to the fact that it is the most implicated serotype in cervical cancer cases, nonetheless, there could be other high risk HPV types that circulate in the state, which were not detected.

6.4 CONTRIBUTION TO KNOWLEDGE

- The study provided information on the prevalence of HPV 16 in Kogi State to be 10%. This is an important finding because HPV types 16 and 18 account for about 80% of all HPV-associated cervical cancer cases.
- The risk factors that were found for HPV infections in this study include; years of use of oral contraceptive, family history of cervical cancer. Sexual behavior and socio demographic factors found to have a statistically significant association with the HPV 16 IgG antibodies are highest level of education and occupation.
- This study was also able to relate the seroprevalence of HPV 16 IgG antibodies with hematological parameters (immune cells) in Lokoja, Kogi State. Until now, there was no such data relating the results of the seropositive women and hematology in the Study. We found an association between the virus and some immune cells. The seropositive women had higher white blood cell counts, lymphocytes, neutrophils and monocytes than the seronegative women. These are 80% as against 64.9% in WBC count, 95% as against 0.8% in lymphocyte count, 87.5% as against 41.6% in monocyte count, 82.5% as against 0.6% in neutrophil count. A change was observed in the pattern inPCV, where 8.6% of

the seronegative women had higher count while 2.5% of the seropositive women had high counts and also in basophil with 5% as against 4.7% and eosinophil with 12.5% as against 16.7%.

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APPENDICES

APPENDIX I: Structured Questionnaire

Department of Microbiology

Faculty of Life Sciences

Ahmadu Bello University, Zaria.

Research Questionnaire

Topic: “Seroprevalence of Human Papillomavirus among Pregnant Women Attending Some Hospitals in Kogi State, Nigeria.”

Introduction : This questionnaire is aimed at getting some socio-demographic and clinical data from you. Your perception is paramount to this research work, as it stands to be basic a background for the consequent judgment and conclusion of my thesis. All the data you will provide is solely for the purpose of this research, hence will be treated in confidence. I therefore solicit with you to be objective and truthful to your mind in filling and answering the questions served below.

Serial no..... Hospital no.....

Instruction : Please tick as appropriate and provide answers where necessary.

Section ‘A’ Personal Data

Age

Marital Status : Single [] Married [] Divorced [] Widowed [] Separated []

Highest level of education : Primary [] Secondary [] Tertiary [] [] Not educated []

Occupation :

Section ‘B’ : Epidemiological Risk Factors

Type of marriage : Monogamy [] Polygamy []. Others specify.....

Number of pregnancies.....`

Trimester of pregnancy.....

Number of children (if any)

Age at first childbirth

Lifetime history of multiple (more than one) sexual partners. Yes [] No []

Do you use contraceptives? Yes [] No []. If yes, what type and for how long?

Do you smoke? Yes [] No []. If yes, for how long?

Age at first sexual contact

Consistent use of condom during sex Yes [] No []

Have you done Pap smear before? Yes [] No []. If yes, state if it was normal or abnormal

History of infection with other sexually-transmitted organisms/diseases, HIV []

Herpes Simplex Virus type 2 (HSV-2) [] Chlamydia [] Syphilis []

Gonorrhoea [] Don't know [] Others, please specify

Family history of cancer

Presence of viral warts around the external genitalia Yes [] No []

APPENDIX II: Ethical consent from Federal Medical Centre, Lokoja



FEDERAL MEDICAL CENTRE LOKOJA

ETHICAL COMMITTEE

1st September, 2015

FMCL\MED\115\II\199

Dr. Haruna Helen Alewo
Department of Microbiology
Faculty of Science
Ahmadu Bello University
Zaria.

ETHICAL APPROVAL IN RESPECT OF DR. HARUNA HELEN ALEWO

With reference to your application for ethical clearance on a research topic
“SEROPREVALENCE OF HUMAN PAPILLOMA VIRUS AMONG PREGNANT
WOMEN ATTENDING CLINIC IN SOME HOSPITALS IN LOKOJA, KOGI
STATE”

This is to convey ethical approval to you having gone through your proposal and
satisfied the Committee.

Your work will be monitored by **Dr. Kassim Davidson O.** to ensure compliance to
approved protocol.

Thank you.

Dr. Osayande Osawe Osa.
Chairman Ethical Committee.

Chairperson
DR. OSAYANDE OSAWE OSA
MB,BS, Fmcophth

Secretary
ABDULLAHI ABDULRAHAMA
BA hons

Financial Officer
JATTO SHUAIB
Bsc, FCA

KOGI STATE SPECIALIST HOSPITAL

P.M.B. 1146, LOKOJA



Our Ref: _____

Your Ref: _____

Date: 18/08/2015

Haruna, Helen Elewo
Dept. of Micro-Biology,
Faculty of Science,
Ahmadu Bello University,
Zaria

Dear Madam,

**RE: SERO PREVALENCE OF HUMAN PAPILLOMA VIRUS AMONG
PREGNANT WOMEN ATTENDING ANTENATAL CLINIC IN SOME
HOSPITALS IN LOKOJA, KOGI STATE**

The ethical committee having received, studied and considered your application for ethical clearance on the above proposal dated 15/06/2015, is pleased to inform you that approval has been granted for your research.

We wish you the best in your endeavours.

DR. AKPCJARO IKPEN
Chairman,
Ethics & Research Committee

MRS. RHODA YENUSA
Ag. Secretary,
Ethics & Research Committee

Ah. Sule Aliu
KSSH Board Chairman

Dr. B. F. Ehalaye
Ag. CMD

Mr. Onojah, B. U.
Director Administration

APPENDIX IV: Ethical Consent from Kogi State Research and Ethics Committee

Kogi State Health Research Ethics Committee
Ministry of Health, P.M.B 1068, Lokoja



H/KGS/1376/1/65

Date 24/7/

ETHICAL CLEARANCE CERTIFICATE

This is to certify that the methodology being adopted

By
HARUNA HELEN ALEWO

For the study of
*PREVALENCE OF HUMAN PAPILLOMAVIRUS AMONG PREGNANT WOMEN ATTENDING ANTENATAL CLINIC IN
HOSPITALS IN LOKOJA, KOGI STATE*

Will not in any way impinge on the ethical standard of medical practice in kogi state, Nigeria


DR. EJEH U.C

Secretary Ethical committee.