Prenatal Maternal Prevalence and New-born Vertical Transmission of Human Papillomavirus (HPV) at the University of Ilorin Teaching Hospital, Ilorin, Nigeria

A. Nyamngee,[2]F.O. Onoriasakpobare, [1]O.O. Agbede, [1]A.A. Akanbi II and [1]M.K. Sulaiman

1.Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Health Sciences, University of Ilorin, Ilorin, Nigeria. 2.Department of Microbiology, University of Ilorin Teaching Hospital, Ilorin.

Abstract

Vertical transmission of Human Papillomavirus (HPV) infection can occur as demonstrated by the established association between a maternal history of genital warts and the development of laryngeal papillomas in children less than 2 years of age. Also there has been reported cases of infants who had genital warts present at the time of delivery. The prevalence and transmission rates of HPV at birth are not known.

This study determined the prevalence of HPV DNA in the cord blood of the baby at delivery and the maternal cervical sample, and the concordance between the prevalence in the cord blood and in the cervical sample using the Polymerase Chain Reaction (PCR).

Cord blood samples were collected at delivery from 113 participating pregnant women, from whom their cervical swab samples had been taken in the antenatal clinic. The specimens were analysed for HPV DNA using the PCR with the consensus primers MY09/MY11.

HPV DNA was detected in 54 cord blood and 11 cervical samples. There were 9 positive concordances for both the cord blood and the cervical sample. The prevalence of HPV DNA in the cord blood was 47.92% and in the maternal cervical sample was 9.7% and the difference was statistically significant. The typing on the positive HPV DNA shows 50.77% positive for HPV 16 and 16.92% positive for HPV 18.

The detection of HPV DNA in the cord blood and the type specific genotypes concordance in the cervical suggests that the mother is the most probable source of HPV positive in the new born. Therefore, this should be a compulsory procedure at the point of delivery for early detection and treatment of HPV infections among the newborn babies.

Key Words: Parental, Prevalence, New-born, Transmission, Papillomavirus.

Correspondence to:

Dr. Nyamngee Amase
Department of Medical Microbiology and Parasitology,
Faculty of Basic Clinical Sciences
University of Ilorin, Ilorin, Nigeria.
Email- anyamngee2010@yahoo.com;
nyamnge.a@unilorin.edu.ng
+2348032110683

Introduction

Human papillomavirus (HPV) is a relatively small, non-enveloped virus with icosahedral symmetry and approximately 50-60nm in diameter. It belongs to the family–Papillomaviridae, and contains a double stranded closed circular DNA genome and protected by a capsid formed by two late proteins (L1 and L2). HPV is the most common sexually transmitted infection among humans, and represents a well-established cause of cervical cancer in females and a significant factor in the development of anogenital, head, and neck cancers.[1]

A number of investigations have found that HPV infection may also be transmitted by non- sexual routes[2,3,4], as HPV DNA has been detected in cord blood and in reproductive and placental cells, as well as in infants, children, and individuals who have never had sexual intercourse.[2,3] HPV horizontal transmissions through saliva or other contact has been used to explain cases of oral infection in infants whose mothers are HPV negative[4] Transient HPV detection in children and conflicting reports on vertical transmission of HPV are inconclusive evidences which need further investigations. Theoretically, vertical transmission can occur as a periconceptual transmission (during fertilization of an oocyte or immediately after fertilization), prenatally (during pregnancy), or perinatally (during or immediately after birth).

Although HPV DNA has been detected in different sites of the male reproductive tract, sperm cells semen endometrium, and ovaries which suggests that HPV could be transmitted during the fertilization of an oocyte or immediately afterward, the significance of these findings is still unknown.[3,4] In vitro analyses have indicated the viability of HPV infection in spermatozoids and the transcription of HPV genes in fecund oocytes[5] and the transcriptional activity of HPV-16 in sperm was confirmed in vivo.[6]

The observation of infants showing signs of HPV-induced lesions at birth, such as laryngeal and anogenital lesions, has led to the belief that intrauterine HPV transmission can occur. HPV DNA has been detected in amniotic fluid, placenta, and the umbilical cord. Both chorionic and placental tissue can be infected through the hematogenous route and hence, HPV can be spread to amniotic cells that are then ingested by the fetus.[6]Transplacental infection, another possible means of HPV intrauterine transmission, can occur through the ascending route from the maternal

https://dx.doi.org/10.4314/tjhc.v29i1.1

genital tract, as it has been shown that the presence of HPV-DNA, both in amniotic fluid and the umbilical cord is correlated with cervical intra-epithelial lesions in pregnant women.[2]

Perinatal transmission is considered the result of the fetus coming into contact with infected cells of the vagina and cervix during birth.[3,4] Some authors have demonstrated that there is both an increased rate of HPV detection among newborns by vaginal delivery (51.4%), compared to those delivered by cesarean section (27.3%) and an increased incidence of juvenile respiratory papillomatosis after prolonged delivery (>10 hours).[6,7] At the same time, others observed a low potential for viral transmission to the oropharyngeal mucosa of newborns from mothers without changes in oncotic colpocytology or a history of genital warts.[8,10,12]

A number of investigations have found that HPV infection may also be transmitted by non-sexual routes, as HPV DNA has been detected in cord blood in reproductive and placental cells, as well as in children, and individuals who have never had sexual intercourse.[2,3] The observation of infants showing signs of HPV-induced lesions at birth, such as laryngeal and anogenital lesions, suggested possible transmission in-utero. Moreso, HPV DNA has been detected in amniotic fluid, placenta, and the umbilical cord.[8] Chorionic and placental tissue can be infected through the hematogenous route and hence, HPV can be spread to amniotic cells that are then ingested by the fetus.[8]

This study investigate the prenatal prevalence and transmission of HPV among new born and their mother pairs, which has been long overdue due to records of high prevalence of HPV infection among women and the possibility of transmission to their new born.

Materials and Methods

The Study Area

The study was conducted at the University of Ilorin Teaching Hospital, a tertiary institution located in Ilorin Kwara State, North-Central geopolitical zone of Nigeria.

The University of Ilorin Teaching Hospital serves as a referral centre for patients not only within Kwara state but also for neighboring states like Niger, Kogi, Ekiti, and Osun states. In addition, even as a tertiary health care centre, it provides primary and secondary health care services.

Study Population

The study population was all pregnant women attending antenatal care and who are in the third trimester of their pregnanciesand are attending the Obstetrics and Gynecology unit of the University of Ilorin Teaching Hospital, Ilorin. Where a cross sectional, hospital-based study was designed and used.

Sampling Techniques

Systematic sampling, where the first participant was randomly selected and others were systematically selected through a predetermined sampling interval was used. Every number that end with and even number was selected to participate in the research.

Sample Size Determination

A total sample size of 113 pregnant women at delivery and their new-born babies were recruited for this study according to Araoye.[9]

Inclusion Criteria

All pregnant women due for delivery attending antenatal clinic and had consented to the study participated in this study. However those whose with gestational age less than 37 weeks were not involved in the study.

Sample Collection

A 2ml of cord blood and cervical specimen was collected from participating pregnant women at delivery and their newborn babies at the UITH using a sterile universal container. These samples were then taken to the Virology Laboratory (HBV/HLA) of the Central Research Laboratories in the University of Ilorin, and stored at -20°C until the DNA was extracted for further analysis.

Procedure for Collection of Cord Blood

The cord blood was collected using the syringe method. Following the delivery of the newborn, the cord was double clamped and cut in between clamps. An empty syringe with a mounted needle was used to extract 2ml of the blood from the umbilical vein of the remaining cord attached to the placenta. The blood collected was immediately transported into a universal sterile container and processed.

Procedure for Collection of Cervical Sample

Cervical specimens were collected with the cervical brush and stored in 90% ethanol at term before onset of labour after counseling and written consent. The Patients were put in dorsal position in the presence of a chaperone and the perineum was exposed with the other regions of the body properly covered. A lubricated cusco's speculum was inserted into the vagina with a gloved hand. The blades were opened and the screw tightened to stay in place, exposing the cervix.

DNA Extraction

The DNA was extracted using "Favorgen DNA Extraction Kit"where the kit applies the unique binding buffer which rapidly lyse cell and inactivate cellular nuclease. The DNA was selectively adsorbed to

silicified membrane in high salt solution. Cellular metabolite and proteins were removed by series of elution-centrifugation steps. Finally, purified DNA from membranes was eluted by low salt elution buffer.

Qualitative Analysis of Extracted DNA

The quality of the genomic DNA was examined by gel electrophoresis using 0.8 % agarose gel. 5µl of each DNA sample was mixed with 1µl of 1X DNA loading dye (1X loading dye consists of 4.16 mg bromophenol blue, 4.16 mg xylene cyanol and 0.66g sucrose in 1ml water) and was loaded into the gel. Electric current was applied at 50 volts until DNA moved into the gel and was raised to 80 volt for rest of the run. Electrophoresis was stopped when the dye had travelled nearly two-third of the gel. Gel was visualized by a Gel doc system (InGenius 3) under UV light and picture was captured.

Agarose Gel Electrophoresis

A 1.5g of agarose gel was weighed with a weighing balance and poured in a conical flask and mixed with 100ml of Tri Acetate EDTA buffer.The conical flask was heated in a microwave until the powder was completely dissolved and the gel, allow cooling to a temperature tolerated by the cheek. Thereafter, 1.5µl of SYBR safe solution was added and rocked gently for the solution to mix properly. The mixture was poured into a gel casting glass which contains two combs placed at distance apart and allowed to solidify, afterwards, the combs were removed gently and cast was placed in the electrophoresis tank and TAE buffer was poured to cover the cast. About 1µl of loading buffer was mixed with 5µl of amplicon, in a loading tray and dispensed into the various wells in the gel. 5µl of the ladder was put in the first well, with 5µl of amplicons each loaded in the next two wells containing a positive and negative control respectively. The gel was run at 100volts for 40mins and care was taken not to run the amplicons off the gel. The result was viewed using a trans-illuminator and the expected base pair of 450bp was compared with that of the ladder or marker to check for positives. Genotyping was done using HPV16 specific primers.

Data Analysis

Data obtained from the result of the laboratory analysis and proforma were analyzed using SPSS (Statistical Package for Social Sciences, version 20, California, USA) to determine the prevalence of HPV DNA in the cord blood and in the maternal cervical sample and also to compare the concordance between the genotype in the cord blood and cervical sample of the selected population.

Results

Demographic Characteristics of Participants

A total of 113 pregnant women who attended the antenatal clinic at the University of Ilorin Teaching Hospital, Ilorin participated in this study. Cervical

Table1. Demographic Characteristics of the Participants

No. of Pregnancy	HPV+	HPV-	X^2	DF	P-value
1	3 (2.7%)	26 (23.0%)	1.558	4	0.816
2	4 (3.5%)	53 (47.8)			
3	3 (2.7%)	17 (15.0%)			
4	1 (0.9%)	5 (4.4%)			
Age of Mothers					
15-24	3 (2.7%)	41 (36.3%)	1.638	1	0.441
25-34	5 (4.4%)	47 (41.6%)			
35-44	3 (2.7%)	14 (12.4%)			
Gender of New born					
Male	29 (25.7%)	37 (32%)	0.942	1	0.332
Female	25 (22.1%)	22 (19.5%)			
Type of Marriage					
Monogamy	10	17	0.347	1	0.556
Polygamy	1	5			
Marital Status					
Single	0	1	0.109	1	0.742
Married	11	101			
Educational Level					
Primary	2	21	1.053	2	0.91
Secondary	5	58			
Tertiary	4	23			

Table 1 shows demographic characteristics of newborn and mothers infected with HPV DNA. Three out of 29 mothers who have only been pregnant once had H PV DNA detected in their cervix, while one out of six mothers had HPV DNA and have been pregnant four times.

Table 2: Prevalence of HPV DNA in the Cord Blood and Cervical samples

	HPV+	HPV-
Cord Blood	54 (47.8%)	59 (52.2%)
Cervical Samples	11 (9.7%)	102 (90.3%)
Total	**65**	**161**

Table 2 above shows the prevalence 47.8% and 9.7% of HPV DNA prevalences of cord blood and cervical samples respectively.

Table 3: HPV Concordance between maternal cervical samples and cord blood

Mother (Cervical Sample)	**New-born (Cord Blood)**	
DNA	**HPV+ (%)**	**HPV- (%)**
HPV+	9 (8.0%)	2 (1.8%)
HPV-	45 (39.8%)	57 (50.4%)

X^2=5.656, df= 1, p-value=0.017

Table 3 shows HPV concordance between maternal cervical and cord blood samples. Nine mothers and their newborns had HPV DNA detected in their samples while two mothers had HPV DNA and their newborns had no detectable HPV DNA.

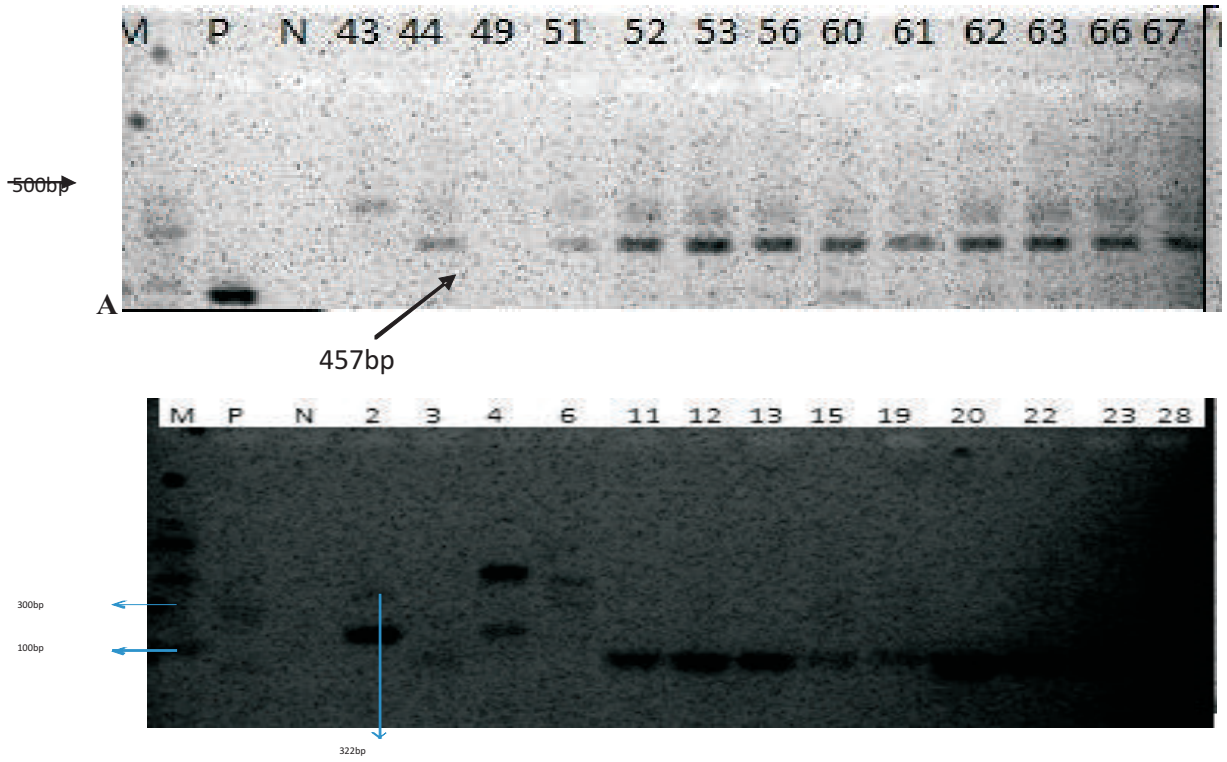

Figure 1: Agarose gel showing HPV 16 positive(A) and HPV 18 positive (B).
The 'A' shows lane M-100bp DNA marker, lane P-Positive control and lane N- Negative control. Lanes 28-39 shows HPV 16 positive bands at 457bp and the 'B' shows lane M-100bp DNA marker, lane P- Positive control and lane N-Negative control. Lanes 2 and 6 showed positive bands at 322bp with HPV 18 primers.

samples were collected from these participants at the antenatal clinic and their corresponding cord bloods were collected at delivery. The minimum age of the participants was 19 years and the maximum age was 42 years. The mean age of participants was 27.02 as shown in Table 1below.

Table 4: Concordance between Maternal cervical samples and cord blood for HPV16 and HPV 18

Cervical samples	Cord blood (No. %)	
DNA	**HPV 16+**	**HPV16-**
HPV 16+	3(4.61)	4 (6.15)
HPV16-	23 (35.38)	32 (49.23)
DNA	**HPV 18 +**	**HPV 18-**
HPV 18+	0 (0.00)	2(3.10)
HPV 18-	7 (10.81)	56 (86.15)

Table 4 above shows the concordance between mother's cervical samples and cord blood samples of newborns for HPV 16. Three mothers and their newborns had HPV 16 DNA detected in their samples while four mothers had HPV 16 DNA and their newborns had no detectable HPV 16 DNA. For HPV 18, there was no concordance between mothers and their newborns while two mothers had HPV 18 DNA and their newborns had no detectable HPV 18 DNA.

Prevalence of HPV DNA

A total of 11 cervical samples were positive for HPV DNA and 54 cord blood samples were positive for HPV DNA (Table 2).The prevalence of HPV DNA in the mother and the new born are 9.7% and 47.8% respectively.

HPV DNA Concordance Between Mother and Newborn

From the total number of mothers and new born positive for HPV there was HPV DNA concordance in 9 (8%) newborns and their mothers while only 2 HPV DNA positive mothers had negative babies (Table 3)

Genotyping for HPV 16 and 18 as well as the Concordance in Mother and Child

Mother and newborns positive for HPV DNA were typed for HPV 16 and HPV 18 using HPV 16 and HPV 18 specific primers and control. HPV 16 regarded as positives when seen on agarose gel with an approximate band size of 457 base pair corresponding to the positive control and HPV 18 was seen at 322 base pair corresponding to the positive control. A total of 33 samples were positive for HPV 16. Among which 26 were cord blood samples and 7 were cervical samples and there were 3 concordances between the cord blood and the cervical samples. A total of 9 HPV DNA was positive for HPV 18, among these positives for HPV 18 DNA, 7 were cord blood and 2 were cervical samples. There was no concordance between the HPV 18 DNA of the cord blood and cervical samples (Table4)

Discussion

The findings from this study shows that HPV DNA positivity of cord blood was highest in male (25.7%) than in female (22.1%), which is similar to the findings of[10]. where sero-positivity to HPV was more prevalent among boys than girls but the reason for this difference remains unclear. The positivity to HPV was compared with number of pregnancies but the difference was very minimal. This implies that HPV transmission may be more related to sexual activities and not influenced by number of pregnancies. Also, the age distribution of HPV in mothers was determined and the prevalence was higher between the mothers of 25 and 34 years which are expected to be the period of active sexual life.

This study further suggested that Human papillomavirus can be vertically transmitted during pregnancy as HPV DNA was detected in the cord blood specimen. The prevalence of HPV DNA in the cord blood was 47.92% and in the maternal sample was 9.7%. The concordance between the HPV DNA in cervical and cord blood samples suggests that the HPV-infected mother is the most probable source of HPV infection of the new born. This view is supported by [11, 12]. who reported vertical transmission rates between 18.2% and 53.3% in the mother-baby pairs respectively.The result from this study also shows 39.8% of HPV positive for the cord blood of the new born and negative to the maternal genital sample which is in line with the work of [10]. He showed that HPV detection in the cord blood or placenta was associated with a history of productive genital HPV infection but not with maternal HPV DNA detection before delivery as HPV may have infected the placental at an earlier stage of pregnancy and could have cleared from the cervix by the time of delivery.

Furthermore, 4.61% of HPV 16 infections were in concordance with both maternal and new born HPV DNA and 3.07% shows multiple infections with HPV 18 which is in line other findings.[13] Smith detected pair concordance of HPV types in only one maternal-infant pair whereas one-third of new born of negative mothers tested positive. Also in 1992, Tseng and colleagues using PCR with type specific primers for HPV 16 reported the presence of HPV DNA in maternal peripheral blood lymphocytes and cord blood and suggested that exposure of infants to HPV during vaginal delivery may be common, and they also noted that HPVDNA in umbilical cord blood was more closely related to the status of HPV DNA in maternal

peripheral blood samples and maternal cervico-vaginal cells. [14]

This finding also shows the possibility of HPV transmission during fertilization as 39.8% of new born were positive for HPV DNA and the corresponding mothers were negative for HPV DNA. Also, HPV typing shows that, 35.38% and 7% were positive for HPV 16 and HPV 18 respectively and negative to the mother which suggests that transmission might have occurred during fertilization and not present at the cervix during cervical sample collection. Other studies[15, 16, 17] also detected HPV DNA in different sites of the male reproductive tract, sperm cells, semen, endometrium and ovaries and suggested that HPV could be transmitted during the fertilization of an oocyte or immediately afterward.

HPV 16 and HPV 18 were detected in the cord blood of the new born and these are high risk HPV which have been shown to be associated with anogenital cancers and also implicated in cancers of the penis, anus, vulva and vaginal, as well as in squamous cell cancers of the conjunctiva, mouth, oropharynx, tonsils and larynx. Studies have shown HR HPV DNA in 15% of genital mucosa of new born and 16% of pregnant women at third trimester of pregnancy and a slight decreased after delivery and first month of follow-up. [18,19, 20, 21] However, he observed an increased in genital HPV at the age of 6 months which he suggested might be due to diminished protection by maternal antibodies and which can also result in persistent HPV infection.

Conclusion and Recommendation

The prevalence of HPV DNA is high in the cord blood using the polymerase chain reaction (PCR) detection method. The detection of HPV DNA in the cord blood and the type specific genotypes concordance suggest that the mother is the most probable source of HPV positive in the new born. Therefore, further investigated to fully understand its occurrence, risk factors and possible implications should be undertaken. It is recommended that this procedure should be made a routine and compulsory to all newborns and their mothers at the point of delivery for early detection and treatment of HPV infection among the newborn babies to reduce the infant mortality rate.

References

1. WHO/ICO Information Centre on HPV and Cervical Cancer (HPV Information Centre). Human papillomavirus and related cancers in the world: summary report. Available at: http://www.who.int/hpvcentre. Accessed 26 September 2011.

2. Syrjanen S. Current concepts on human papillomavirus infections in children. APMIS 2010; 118:494–509.

3. Garolla A, Pizzol D, Foresta C. The hole of human papillomavirus on sperm function. Current Opinion Obstetric Gynecology 2011; 23:232–7.

4. Foresta C, Patassini C, Bertoldo A, et al. Mechanism of Human Papillomavirus binding to Human Spermatozoa and Fertilizing Ability of infected Spermatozoa. PLoS One 2011; 6:15036.

5. American College of Obstetricians and Gynecologists. (2000). "Make Decisions about Human Papillomavirus Based on Sound Medicine, Rather than Politics."

6. Anic, Gabriella M. and Anna R. Giuliano. (2012)."Genital HPV infection and related lesions in men."*Preventive Medicine*, 53 (Supp 1): S36–S41.

7. Bodily J. & Laimins, LA. (2011). Persistence of human papillomavirus infection: keys to malignant progression. *Trends in Microbiology*, Vol. 19, No. 1, (Jan 2011), pp. 33-39,ISSN 0966-842X.

8. Centers for Disease Control. (2013). *Ready-To-Use STD Curriculum - Human Papillomavirus(HPV) Module – Faculty Notes*. [Online]. http://www2a.cdc.gov/stdtraining/ready-to-use/Manuals/HPV/hpv-notes-2013.pdf, accessed December 2, 2014.

9. Araoye, M. Research Methodology with statistics for health and social sciences. Nathadex publishers, Ilorin, (2003).pp 118.

10. Centers for Disease Control. (2014). *Genital HPV Infection – Fact Sheet.* [Online]. http://www.cdc.gov/std/HPV/STDFact-HPV.htm, , accessed 2014, December 2.

11. Schiller, JT., Day, PM. & Kines, RC. (2010).Current understanding of the mechanism of HPV infection. *Gynecologic Oncology*, Vol. 118 (Jun 2010), Suppl 1, S12-17, ISSN 0090-8258.

12. Timmons, B., Akins, M. & Mahendroo, M. (2010). Cervical remodeling during pregnancy and parturition. *Trends in Endocrinology Metabolism.* Vol. 21, No 6, (June 2010), pp. 353–361, ISSN 1043-2760.

13. American Cancer Society. (2015). *Cervical Cancer*. Atlanta, GA: American Cancer Society, Inc

14. González, P., Hildesheim, A., Rodríguez, AC., Schiffman, M., Porras, CP., Wacholder,

S.,Piñeres, AG., Pinto, LA., Burk, RD & Herrero, R. (2010). Behavioral/lifestyle and immunologic factors associated with HPV infection among women older than 45 years. *Cancer Epidemiology. Biomarkers & Prevention*, Vol. 19, No. 12, (Dec 2010), pp, 3044-3054, ISSN 1055-1065.

15. Moody, CA. & Laimins, LA. (2010). Human papillomavirus oncoproteins: pathways to

transformation. *Nature Reviews Cancer*, Vol. 10, No. 8, pp. 550-560, ISSN 1474-175X.

16. Kanodia S, Fahey LM, Kast WM. Mechanisms used by human papillomaviruses to escape the host immune response. *Current Cancer Drug Targets*. Vol. 7, No. 1 (Feb 2007), pp.79-89, ISSN 1568-0096.
17. National Cancer Institute. (2014). *Cervical Cancer Prevention* (PDQ®). [Online]. http://www.cancer.gov/cancertopics/pdq/prevention/cervical/HealthProfessional, accessed December 2, 2014.
18. Stanley MA. (2010). Pathology and epidemiology of HPV infection in females. *Gynecologic Oncology*, Vol. 117, Suppl 2, (May 2010), pp. S5-10, ISSN 0090-8258.
19. American Social Health Association (ASHA). (2014) *Genital Warts.*
20. Lazarczyk, M., Cassonnet, P., Pons, C., Jacob, Y. & Favre, M. (2009). The EVER proteins as a natural barrier against papillomaviruses: a new insight into the pathogenesis of human papillomavirus infections. *Microbiology and Molecular Biology Reviews,* Vol.73, No. 2, (June 2009), pp. 348-370, ISSN 092-2172.
21.Terese E. Azua, Priscilla Y. Orkaa, Regina A.I. Ega. Prevalence of Human Papillomavirus(HPV) infection among pregnant women attending antenatal care at General hospital, minna, Nigeria. Journal Microbial Biotech. Research., 2015, 5 (3): 38-44