



Detection of HPV DNA in Cord Blood of Newborns in Ilorin, Nigeria

Onoriasakpobare, F. O.

Department of Microbiology, Dennis Osadebay University, Asaba, Nigeria

Corresponding author email: felix.onoriasakp-obare@dou.edu.ng

Abstract

The contagious virus known as the human papillomavirus (HPV) can proliferate within live cells. The virus has icosahedral symmetry and is small. It has a diameter of 50–60 nm. Its double-stranded, closed circular DNA genome is protected by a capsid made up of two late proteins (L1 and L2), and it is a member of the Papillomaviridae family. The most prevalent viral sexually transmitted infection in humans, the human papillomavirus, is known to cause malignancies of the head, neck, anogenital area, and cervical region. At the University of Ilorin Teaching Hospital's prenatal clinic, 113 expectant women were examined. For molecular detection, DNA extraction, and HP-viral screening, MY09/MY11 and GP5+/GP6+ consensus primers were employed. HP-viral DNA was found in 54 (47.8%) of the cord blood samples. Infants' HPV DNA may indicate HPV-related diseases. To avoid transmission during pregnancy, routine monitoring and surveillance are advised for both pregnant and postpartum women, and HPV vaccination is required during family planning before pregnancy.

Keywords: HPV DNA, Newborns, Cord blood, HPV-viral disease, HPV vaccination

Introduction

The human papillomavirus (HPV) is an infectious virus that can multiply inside living cells. It is a little virus with icosahedral symmetry that is not confined. Its diameter is between 50 and 60 nm. It belongs to the Papillomaviridae family and has a capsid composed of two late proteins (L1 and L2) that protect its double-stranded closed circular DNA genome. Head, neck, anogenital, and cervical cancers are known to be caused by the human papillomavirus, the most common sexually transmitted infection in humans. Nonsexual transmission of HPV infections has been demonstrated (Syrjänen, 2010). The finding of HPV DNA in placental cells and cord blood lends credence to this claim. Furthermore, HPV has been detected in babies, children, and non-sexually active adults (Garolla et al., 2011). According to Armbruster-Moraes et al. (1994), Romaldi et al. (2009), and Onoriasakpobare et al. (2024), HPV DNA has been found in the placenta, umbilical cord, and amniotic fluid. Furthermore, as the hematogenous pathway is known to infect both chorionic and placental tissue, HPV can go to amniotic cells, which the growing fetus subsequently consumes (Armbruster-Moraes et al., 1994; Syrjänen, 2010).

Materials and Methods

From August 2017 to June 2018, pregnant women at the Obstetrics and Gynecology unit of the University of Ilorin Teaching Hospital (UIITH), Ilorin, Nigeria, participated in this prospective hospital-based cross-sectional study. To locate willing volunteers, the study employed a sequential non-probability sampling technique. After reading the protocol and providing written informed consent, 113 pregnant women with gestational ages greater than 37 weeks who gave birth vaginally at UIITH took part in the study. Samples of cord blood were drawn on the day of delivery and placed in tubes with EDTA anticoagulant. They were then taken in colder ice boxes to the University of Ilorin's Central Research Laboratories' Virology department, where they were kept at -20°C until examination.

Genomic DNA Extraction of Viral Nucleic Acid from Cord Blood

Following the manufacturer's instructions, a DNA extraction kit (Favorgen, South Korea) was used to extract DNA from the cord blood. Using the MY09/11 and GP5+/6+ consensus primers, HPV detection was carried out in a nested PCR with minimum modifications, by the previously reported procedures of Venceslau et al. (2014). Following an initial denaturation period of nine minutes, forty amplification cycles were carried out for thirty seconds at 95°C. Following the first and nested rounds of amplification, a 2-minute annealing phase was carried out at 50°C and 45°C. After that, there were two extensions at 72°C, one lasting 30 seconds and the other four

minutes. Using InGenius 3™ +System (Syngene, USA), a 450 bp fragment was discovered on a 1.5% agarose gel that had been stained with Sybr safe dye. A Veriti Thermal Cycler (Applied Biosystem, USA) was used for each PCR. The Edvotek electrophoresis equipment (www.edvotek USA) and the InGenius 3™ + equipment (Syngene, USA) were used for the electrophoresis and detection.

Statistical Analysis

Graph Pad Prism 8.02 (San Diego, USA) was used to thoroughly assess the data obtained in this study to get mean, percentage, and chi-square test results. Group differences were assessed using the chi-square test, and a two-sided probability of less than 0.05 was deemed statistically significant.

Results

With a mean age of 27.0, the 113 pregnant women in this study were between the ages of 19 and 42; most participants were in the 25–34 age group (Table 1). About 5.4% (6/112) of the pregnant women were in polygamous marriages, and only one was single. The percentage of women with two pregnancies was higher than the percentage of women with four pregnancies (6/113), at 50.4% (57/113). 54 (47.8%) of the newborn's cord blood samples had HPV DNA.

Demographic Characteristics of Participants about HPV Infection

Features	HPV + (%)	HPV – (%)	χ^2	DF	P-value
Age of Mother (Years)					
15-24	3 (2.7)	41 (36.3)	1.64	2	0.441
25-34	5 (4.4)	47 (41.6)			
35-44	3 (2.7)	14 (12.4)			
Marital Status					
Married	11	101	0.11	1	0.742
Not Married	0	1			
Type of Marriage					
Monogamy	10	96	0.34	1	0.563
Polygamy	1	5			
Sex of Newborn					
Male	29 (25.7)	37 (32.0)	0.94	1	0.332
Female	25 (22.1)	22 (19.5)			

Discussion

The results of the investigation showed that cord blood contained HPV DNA. The presence of HPV DNA in these intrauterine locations was not anticipated by the delivery technique. HPV can cross the placenta and raise the fetus's risk of catching the virus during intrauterine transmission, according to the study's findings. Previous research indicates that the percentage of HPV DNA in cord blood ranges from 0% to 13.5% (Rice et al., 2000). Additionally, in placental material, it ranges from 0 to 42.5% (Puranen et al., 1996; Rombaldi et al., 2009; Smith et al., 2010). The placenta and umbilical cord blood had the lowest HPV detection rates, according to studies that did not include pregnant women with clinical HPV lesions (Rombaldi et al., 2009; Smith et al., 2010). Worda and colleagues (Rombaldi et al., 2009) examined the cord blood, placenta, and amniotic fluid of 153 clinically HPV-negative moms having cesarean sections, which was the biggest cohort before the current study. 5.2% of the placental samples revealed high-risk HPV, whereas all cord blood and amniotic fluid samples tested negative for HPV. Following the discovery that 4.2% of placentas and 3.5% of cord blood samples were positive for HPV, the study's cohort size was expanded. We would have had the same exclusion criteria as Worda and colleagues, except delivery mode, which would have led to HPV detection rates of only 2.7% (6/225) and 1.8% (4/224), respectively. The contradictory results could be due to sampling techniques. Even distribution of viral DNA throughout the placenta is very rare. Only a small portion of the placenta is commonly tested for HPV, and the typical villous area of a placenta at 40 gestational weeks is 125,000 cm² (Vencesian et al., 2004). Because some HPV-positive placentas would undoubtedly be overlooked, the true rate of transplacental transmission may be significantly higher than currently reported. It is always possible for samples taken in an operating area or delivery room to become contaminated. However, to precisely detect HPV in placental syncytiotrophoblastic cells, we employed an incredibly sensitive ISH in our investigation. Positive syncytiotrophoblast results, which show that HPV DNA is present in these placental cells, show that the presence of HPV in placental samples is not due to contamination of the birth canal surface. This confirms the results of Hermonat and associates (Wang et al., 1998), who examined tissues from spontaneous miscarriages using the in situ PCR technique and discovered HPV DNA in syncytiotrophoblast nuclei. As far as we are aware, this is the first study to demonstrate that the

syncytiotrophoblast nuclei from healthy placentas obtained after full-term infants contain HPV DNA. Recent studies suggest that HPV16, like HPV11, 18, and 31, may complete its life cycle in the trophoblast cell line 3A (Sarkola et al., 2008; Silverberg et al., 2003). Thus, our findings lend credence to the idea that HPVs are not just keratinocyte-specific.

In 13.5% (7/52) of cord blood samples obtained at delivery, Tseng and associates found HPV16 DNA (Rice et al., 2000). Because the level of HPV DNA in cord blood was more closely correlated with the level of HPV DNA in maternal peripheral blood mononuclear cells (PBMC) than in maternal cervicovaginal cells, we were unable to confirm the conclusion that HPV infection among cord blood HPV-positive neonates most likely represents viral infection in utero through trans-placental transmission. None of the PBMC samples from the moms who had an HPV placenta or a newborn with HPV cord blood tested positive for HPV DNA at the time of study enrollment, which takes place in the final month of pregnancy. Since the date and duration of a potential viremic phase of HPV are entirely unknown, it is challenging to estimate the potential impact of this gap on the results. Cord blood HPV positivity was found to be independently predicted by the prevalence of maternal genital warts. Furthermore, Pap screen abnormalities identified before birth were linked to placenta and cord blood samples that tested positive for HPV. These findings suggest that a successful vaginal HPV infection may be linked to HPV detection in the placenta or cord blood. Because high concentrations of viral particles can contaminate the vaginal tract mucosa, the presence of HPV DNA in the placenta and amniotic fluid increases the risk of an ascending intrauterine infection (Koskimaa et al., 2017). Furthermore, due to particular genetic variables or altered immune responses, individuals with visible clinical lesions and productive infections may also be more vulnerable to HPV survival and transmission in general. Since it is currently unable to measure the viral load from these women, this theory needs to be verified. It's interesting to note that placenta or cord blood HPV positive was typically unrelated to the HPV detected in the mother's prenatal oral or vaginal scrapings. This may result from improper negative DNA scraping or insufficient sampling. On the other hand, HPV might have left the cervix after entering the placenta early in pregnancy. We have demonstrated that HPV DNA clearance occurs 12 months before the decline in Pap smear abnormalities, which explains why Pap smear abnormalities are frequently detected in these women before delivery (Watts et al., 1998). The infant was more likely to test positive for HPV at birth if HPV DNA was detected in the placenta or cord blood. Generally speaking, the neonate's HPV status could not be predicted by the method of birth. This is consistent with past research demonstrating that neonates are not protected from HPV by cesarean deliveries (Caglar & Garrido, 2018). It will be determined by the children's long-term follow-up if the early HPV exposure results in natural immunity or if these newborns who test positive for HPV right after delivery are more likely to have clinical HPV lesions later in life. Additionally, long-term monitoring will verify whether more comprehensive monitoring (such as HPV testing) of infants born to women infected with HPV is required. To the best of my knowledge, persons with HPV placenta or HPV cord blood have never before had their risk of HPV transmission from mother to child evaluated.

Conclusion

The detection of Human Papillomavirus (HPV) DNA in cord blood is a significant finding that suggests that HPV DNA can be transmitted from mother to child during pregnancy or childbirth, highlighting the importance of prenatal screening and management, its also suggest that newborns may be exposed to HPV, which could potentially lead to early infection and increased risk of developing HPV-related diseases later in life.

Recommendations

1. Pregnant women should be screened for HPV to identify those at risk of transmitting the virus to their newborns. Pregnant women who are not already vaccinated against HPV should receive the vaccine after pregnancy to protect themselves and their future children.
2. Newborns exposed to HPV in utero or during childbirth should be monitored for signs of HPV-related diseases.

Acknowledgement

Throughout patient recruitment and specimen collection, the author received significant support from the resident physicians and nurses at the Obstetrics and Gynecology unit of the University of Ilorin Teaching Hospital (UITH), Ilorin.

Source of Funding:

No funding sources were supporting this work.

Compliance with Ethical Standards

This study was approved by the Ethics Review Committee of the University of Ilorin Teaching Hospital and was conducted in compliance with the Helsinki Declaration (ERC PAN/2018/06/1790). Every participant gave their written informed consent during the study.

Consent to Publish

The authors attest that participants in the human study gave their informed consent before their data could be published in a respectable journal.

Conflicts of Interest

There are no conflicts of interest to disclose, according to the author.

References

- Armbruster-Moraes, E., Ioshimoto, L. M., Leão, E., & Zugaib, M. (1994). Presence of human papillomavirus DNA in amniotic fluids of pregnant women with cervical lesions. *Gynecologic oncology*, *54*(2), 152-158.
- Çağlar, G. S., & Garrido, N. (2018). The implications of male human papillomavirus infection in couples seeking assisted reproduction technologies. *Journal of the Turkish German Gynecological Association*, *19*(1), 48.
- Garolla, A., Pizzol, D., & Foresta, C. (2011). The role of human papillomavirus on sperm function. *Current Opinion in Obstetrics and Gynecology*, *23*(4), 232-237.
- Koskimaa, H.-M., Paaso, A., Welters, M., Grénman, S., Syrjänen, K., Van Der Burg, S., & Syrjänen, S. (2017). The presence of human papillomavirus (HPV) in placenta and/or cord blood might result in Th2 polarization. *European journal of clinical microbiology & infectious diseases*, *36*(8), 1491-1503.
- Onoriasakpobare, F. O., Ashaka, O. S., Omoare, A. A., Jimah, E. M., Igere, B. E., Omamuyovwe, P., ... & Agbede, O. O. (2024). Prenatal Transmission of High-Risk HPV 16 And HPV 18 Among Antenatal Mothers In Western Nigeria. *Current Research in Interdisciplinary Studies*, *3*(4), 17-23.
- Puranen, M., Yliskoski, M., Saarikoski, S., Syrjänen, K., & Syrjänen, S. (1996). Vertical transmission of human papillomavirus from infected mothers to their newborn babies and persistence of the virus in childhood. *American journal of obstetrics and gynecology*, *174*(2), 694-699.
- Rice, P. S., Mant, C., Cason, J., Bible, J. M., Muir, P., Kell, B., & Best, J. M. (2000). High prevalence of human papillomavirus type 16 infection among children. *Journal of medical virology*, *61*(1), 70-75. 1011
- Rombaldi, R. L., Serafini, E. P., Mandelli, J., Zimmermann, E., & Losquiavo, K. P. (2009). Perinatal transmission of human papillomavirus DNA. *Virology journal*, *6*(1), 1-12.
- Sarkola, M. E., Grénman, S. E., Rintala, M. A., Syrjänen, K. J., & Syrjänen, S. M. (2008). Human papillomavirus in the placenta and umbilical cord blood. *Acta Obstetrica et Gynecologica Scandinavica*, *87*(11), 1181-1188.
- Silverberg, M. J., Thorsen, P., Lindeberg, H., Grant, L. A., & Shah, K. V. (2003). Condyloma in pregnancy is strongly predictive of juvenile-onset recurrent respiratory papillomatosis. *Obstetrics & Gynecology*, *101*(4), 645-652.
- Smith, E. M., Parker, M. A., Rubenstein, L. M., Haugen, T. H., Hamsikova, E., & Turek, L. P. (2010). Evidence for vertical transmission of HPV from mothers to infants. *Infectious diseases in obstetrics and gynecology*, *2010*.
- Smith, E. M., Ritchie, J. M., Yankowitz, J., Swarnavel, S., Wang, D., Haugen, T. H., & Turek, L. P. (2004). Human papillomavirus prevalence and types in newborns and parents: concordance and modes of transmission. *Sexually transmitted diseases*, *57*-62.
- Sotlar, K., Diemer, D., Dethleffs, A., Hack, Y., Stubner, A., Vollmer, N., ... Wallwiener, D. (2004). Detection and typing of human papillomavirus by e6 nested multiplex PCR. *Journal of clinical microbiology*, *42*(7), 3176-3184.
- Syrjänen, S. (2010). Current concepts on human papillomavirus infections in children. *Apmis*, *118*(6-7), 494- 509.
- Syrjänen, S. (2018). Oral manifestations of human papillomavirus infections. *European journal of oral sciences*, *126*, 49-66.
- Venceslau, E. M., Bezerra, M. M., Lopes, A. C. M., Souza, É. V., Onofre, A. S. C., Melo, C. M. d., . . . Onofre, F. B. d. M. (2014). HPV detection using primers MY09/MY11 and GP5+/GP6+ in patients with

- cytologic and/or colposcopic changes. *Jornal brasileiro de patologia e medicina laboratorial*, 50, 280- 285.
- Wang, X., Zhu, Q., & Rao, H. (1998). Maternal-fetal transmission of human papillomavirus. *Chinese medical journal*, 111(08), 726-727.
- Watts, D. H., Koutsky, L. A., Holmes, K. K., Goldman, D., Kuypers, J., Kiviat, N. B., & Galloway, D. A. (1998). Low risk of perinatal transmission of human papillomavirus: results from a prospective cohort study. *American journal of obstetrics and gynecology*, 178(2), 365-373.
- Worda, C., Huber, A., Hudelist, G., Schatten, C., Leipold, H., Czerwenka, K., & Eppel, W. (2005). Prevalence of cervical and intrauterine human papillomavirus infected in the third trimester in asymptomatic women. *Journal of the Society for Gynecologic Investigation*, 12(6), 440-444.