RESEARCH COMMUNICATION

Human Papillomavirus Infection Among Indian Mothers and Their Infants

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Abstract

Objective: Several studies have demonstrated that infants can acquire human papillomavirus (HPV) infection at birth from their mothers. The aim of the present investigation was to determine prevalence of HPV infection among pregnant women and evaluate the extent of perinatal transmission of HPVs to infants.

Methods: The study included 135 pregnant women and their infants. The polymerase chain reaction (PCR) was performed to detect HPV DNA in cervical cells of the women and buccal cells of the infants.

Results: HPVs detected were genotyped by PCR using type specific primers. HPV DNA was identified in 38 mothers (28.14%, 38/135) and 14 babies (10.37%, 14/135). The prevalence rate of HPV type 16 was highest both in HPV positive maternal (63.15%, 24/38) and baby samples (85.71%, 12/14). At birth, the frequency of HPV transmission from infected mothers to their infants was 18.42% (7/38). The proportion of infants with HPV infection delivered by cesarean section was 78.57% (11/14).

Conclusion: Cesarean section was not found protective for infants against perinatal HPV transmission. Infection in the infants was cleared within one year. This is the first report of its kind from India.

Key Words: Perinatal transmission - infants - HPV infection - pregnant women.

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Introduction

Epidemiological studies have firmly demonstrated that human papillomaviruses (HPVs), are established causes of anogenital cancers (Schiffman,1995, Das et al, 2000, Kirwan & Herrington, 2001). HPV 16 and 18 are the most prevalent types in cervical lesions (Van den Brule et al, 1993). Low risk HPV types 6 and 11, in contrast, are mainly associated with recurrent laryngeal papillomatosis and genital warts (Levi et al, 1989). Though HPV is mainly sexually transmitted, there are reports (Cason et al, 1995) of perinatal transmission leading to subclinical infection in infants. This may occur owing to swallowing of the maternal blood, amniotic fluid or vaginal secretions as the fetus passes through an infected birth canal (Tseng et al, 1998). Frequency of HPV infection among pregnant women (Rando et al, 1989) and recurrent laryngeal papillomatosis (Kashima et al, 1987) and condyloma acuminata (Obalek et al, 1990) in newborn infants support perinatal transmission of HPVs. The majority of HPV infections are transient and persistence of the infection is required for the development of cervical intraepithelial neoplasia (Hildesheim et al, 1994). Acquisition of HPV infection before the infants' immune system is mature, may lead to immunological tolerance. This would facilitate persistence of the infection and/or a reduced ability to clear out subsequent high risk HPV reinfections (Cason et al, 1998). Thus assessment of prevalence and persistence of HPV infection has significant implications for vaccination against genital HPV infection.

We here therefore investigated the prevalence of cervical HPV infection among Indian pregnant women and evaluated the risk of perinatal transmission from mother to infant. The

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objective was to identify a group of women at risk of developing premalignant /malignant cervical lesions at later stage of life and to understand the potential for perinatal HPV transmission in India.

Patients and Methods

Subjects

Between May 1999 and December 2001, 215 pregnant women at term attending antenatal clinic at BNR hospital, Gardenreach, Kolkata and their infants were recruited for this study. This study was commenced after obtaining approval of the institutional ethical board. At enrollment samples were collected from 215 pregnant women in the age range 20- 39 years without any previous history of abnormal cervical smears or visible genital warts and complicated pregnancies. Informed consents for sample collection were received from the women for themselves and their infants. A total of 149 mother-infant pairs were eligible for this study because 66 cases lacking in matched maternal cervical samples and neonatal buccal samples were excluded. Cervical swabs were collected from the women at their third trimester examination 10-15 days prior to delivery. Neonatal buccal swabs were collected between 1-4 days after birth before hospital discharge. Women were asked to return with their infants for further evaluation if either of them were HPV positive at first examination. Interval between enrollment and first return visit was 6 months postpartum for the mothers and 6 weeks for the babies. HPV positive cases were attempted to follow-up to 1 year with an interim visit at 3 months for infants and 6 months for mothers.

Sample collection

To test presence of HPV DNA in exfoliated cervical cells of the pregnant women, cell scrapes were collected with a small endocervical brush and a cervical smear was also taken for cytological analysis. Buccal swabs were collected from infants born to the mothers. All samples were taken in sterile phosphate-buffered saline (pH 7.4) solution and stored at -20^0c until processed.

Detection of HPV DNA

Cells were lysed by proteinase K digestion and nucleic acids were extracted by phenol –chloroform. A 268 bp fragment of the beta globin gene was amplified to assess adequacy of the DNA of each sample (Saiki et al,1988). Negative samples (N=14) from either the mother or the infants by beta globin amplification were excluded from this study. Both samples were adequate in 135 mother – baby pairs. For HPV detection the DNA was amplified using the degenerate consensus primers MY09/MY11 (Manos et al, 1989) targeting a 450 bp region in the highly conserved L1 ORF. PCR products were electrophoresed in ethidium bromide stained 1.5% agarose gel. Positive cases were amplified further with type specific primers for HPVs 6/11 (Ostwald et al,1994), 16,18 (Miller et al, 1994), 31 and 33 (Grce et al, 1997). The length of the final amplified HPV types are 113, 109, 334, 514 and 456 bp respectively. All PCRs included negative controls (distilled water and DNA from HPV negative cell line C33A) and precautions were taken to avoid contaminations. Plasmid DNA containing type specific HPV genome and HPV positive cell lines (SiHa / HeLa) were used as positive controls.

Cytological analysis

Cytological preparations were assessed by standard Papanicolaou staining. The diagnosis of HPV was based on previously reported features (Gupta, 1991). These included parakeratosis, and /or dyskeratosis, keratinization, nuclear enlargement, nuclear membrane irregularities and sharply demarcated paranuclear halo. The cytologist had no prior information about HPV status of the patients.

Statistical calculations

Odds ratio (OR) and Chi- square test was performed to determine statistical significance of different HPV results among the women and to compare the factors in mother associated with HPV transmission between HPV+ and HPV- infants.

Results

The study population primarily comprised of Hindu (123/133, 92.48%) women from lower middle class background (income ranging Rs. 1500 – 5000 per month). Mean age of the patients was 26.55 years (range: 20-39 years). Base information of the subjects is given in Table 1. Overall prevalence of cervical HPV infection among the pregnant women was 28.14% (38/135) at enrollment. Average age of the HPV positive and HPV negative women was 25 and 21 years respectively. Details of the women's characteristics regarding age, social class, cytology results and gravida are given in Table 2.

Table 1. Basic Information for the Study Subjects

variable		percentage
Ethnicity	Hindu	123/133, 92.48
	Others	10/133, 7.52
Mother's age	20-29 yrs	106/135, 78.52
	30-39 yrs	29/135, 21.48
#Social class	I	24/125, 19.2
	II	72/125, 57.6
	III	26/125, 20.8
	IV	3/125, 2.4
*Gravida	1	70/134, 52.24
	2	32/134, 23.88
	3 and/or >3	32/134, 23.88

social class of 10 subjects could not be ascertained.(class IV was highest) *gravida of one subject was unknown

Table 2. Patient Characteristics

Variable	HPV positive	HPV negative	OR
Age (n=135)			
20-29 yrs (106)	25	81	1.00
30-39 yrs (29)	13	16	2.63 (1.12- 6.21)
Gravida (n=134) *			
1 (70)	24	46	3.65 (1.15-11.63)
2 (32)	10	22	3.18 (0.88-11.52)
3 and >3 (32)	4	28	1.00
Cytology (n=135)**			
Normal (94)	15	79	1.00
HPV related lesions(39)	21	18	6.13 (2.66-14.29)
Social class (n=125)#			
I (24)	2	22	
II (72)	27	45	P =0.124
III (26)	8	18	
IV (3)	1	2	

N.B. Number in parenthesis indicates the number of women in that category.

* gravida of one subject was unknown, **insufficient material in 2 samples. #social class of 10 subjects could not be ascertained #class IV was highest

HPV DNA of types 16 and/or 18 was identified in 81.57% (31/38 cases) of the HPV+ women. HPV 16 was detected in 63.15% (24/38) of the women. Out of the 31 HPV positives 13 had HPV type 16, 7 HPV 18 and 11 both HPV 16 and 18. Three of the 13 HPV 16 positives had also HPV 6/11 DNA and one had additionally HPV 31. Among the 7 HPV 18 positive cases, 2 had in addition HPV 31 and one both HPV 31 and 33. Out of the 11 cases having both HPV 16 and 18, 4 were also positive for HPV 6/11, 1 for HPV 31 and one for both HPV 31 and 33. Thus, out of 38 overall HPV positive mothers, 31 had HPV 16 and/or 18, one was infected with both HPV 6/11 and 31 and 6 had HPVs other than the types tested.

Buccal specimen for HPV DNA analysis was collected from all 135 infants (male/female = 63/72). At birth overall prevalence of HPV infection among the infants was 10.37 % (14/135). Among the 14 HPV positive infants, 7 (males / females = 4/3, vaginal /cesarean delivery = 2/5) were born to HPV positive mothers and 7 (males /females = 3/4, vaginal / cesarean delivery = 1/6) to HPV negative mothers. Therefore at birth the overall frequency of HPV transmission from HPV positive mothers to their infants was 18.42 % (7/38). Out of the 14 HPV positive infants 12 had HPV 16 DNA, 1 HPV 31 and 1 HPV 33. Out of the 12 HPV 16 positive babies additional HPV 31 was found in six, HPV 33 in one, HPV 18 plus 31 in one.

Table 3 summarizes the concordance between HPV types in mothers and the infants. It was found that HPV 16 DNA concordantly transmitted in 5 cases regardless of the mode of delivery. None of the mothers selected the mode of delivering their babies. Other than HPV type 16 was found to be transmitted from 14 mothers to 2 babies. However 6 babies were found to acquire HPV 16 infection and 1 other HPV types though they were born of HPV negative mothers. HPV positive mothers and infants were followed up at programmed visits. Mothers were followed up at an interval of 6 months and infants at 6 weeks and 3, 6, 9 or 12 months after delivery. However, all subjects did not turn up as scheduled. Out of 38 HPV positive (at enrollment) mothers 18 were not available for follow up study. At 6 months of follow up 12 mothers were available for study. Out of them 2 remained HPV positive and 10 were negative. At 12-month follow up 8 mothers (excluding previous 12) attended for the test and 3/8 were found to be HPV positive. Types of HPV DNA detected in cervical samples before delivery and at follow up were not identical though prevalence of HPV 16 was highest (data not shown) in both cases. Among 14 HPV positive infants 4 were not available for follow up study. Remaining 10 infants were evaluated at least once, anytime during the follow up periods of upto 12 months. All were found to have cleared the infection in some cases as early as first visit at 6 weeks (data not shown).

Characteristics of mothers that could influence transmission of HPV infection are given in Table 4. It was found that HPV infection in the infants was not associated with the maternal age, socioeconomic level, gravida, HPV

Table 3. HPV Status of the Infants with Respect to Mothers' Status and Mode of Delivery

	Mode of delivery (CS/VD)*	Infants HPV 16	Infants HPV other	Infants HPV negative
Mothers HPV 16 positive	CS (16)	4	-	12
	VD (8)	1	-	7
Mothers other HPV positive	CS (11)	1	-	10
	VD (3)	1	-	2
Mothers HPV negative	CS (49)	5	1	43
	VD (48)	1	-	47

*VD: vaginal delivery; CS : cesarean delivery

Table 4. Characteristics of Mothers and HPV Prevalence in Buccal Cells of the Infants

	Infants HPV Positive	Negative	OR&p
Maternal age (n = 135)			
20-29 yrs mothers'HPV+ (25)	5	20	3.13 (0.73-13.28)
mothers'HPV- (81)	6	75	χ^2 = 3.26, p = 0.0711
30-39 yrs mothers'HPV+ (13)	2	11	2.73 (0.16-87.62)
mothers'HPV- (16)	1	15	χ^2 = 0.04, p = 0.8491
*Social class (n =125)			
I mothers'HPV+ (2)	-	2	χ^2 = 2.37, p=0.1235
mothers'HPV- (22)	1	21	
II mothers'HPV+ (27)	5	22	2.33 (0.47- 11.79)
mothers'HPV- (45)	4	41	χ^2 =0.69, p = 0.4076
III mothers'HPV+ (8)	2	6	2.67 (0.20- 37.04)
mothers'HPV- (18)	2	16	χ^2 = 0.10, p= 0.75118
IV mothers'HPV+ (1)	-	1	
mothers' HPV- (2)	-	2	
#Gravida (n =134)			
1 mothers'HPV+ (24)	4	20	2.10 (0.39- 11.47)
mothers'HPV- (46)	4	42	χ^2 = 0.36, p = 0.54901
2 mothers'HPV+ (10)	2	8	2.5 (0.20 – 31.88)
mothers'HPV- (22)	2	20	χ^2 = 0.08, p= 0.7731
3 and >3 mothers'HPV+ (4)	1	3	
mothers'HPV- (28)	1	27	χ^2 =0.30, p=0.5809
*Cytology (n = 133)			
normal mothers'HPV+ (15)	2	13	2.28 (0.27-15.7)
mothers'HPV- (79)	5	74	χ^2 = 0.17, p= 0.6811
abnormal mothers'HPV+ (21)	4	17	1.88 (0.24 –17.48)
mothers'HPV- (18)	2	16	χ^2 =0.06, p=0.8156
HPV positive by PCR			
mothers'HPV+ (38)	7	31	2.90 (0.83-10.18)
mothers'HPV- (97)	7	90	χ^2 =3.69, 0.0548
mode of delivery			
cesarean mothers'HPV+ (27)	5	22	1.63 (0.38-6.99)
mothers'HPV- (49)	6	43	χ^2 =0.55, p= 0.4568
vaginal mothers'HPV+ (11)	2	9	10.44 (0.63 – 329.12)
mothers'HPV- (48)	1	47	χ^2 =4.81, p= 0.02836

*social class of 10 subjects could not be ascertained.
#gravida of one subject was unknown; ** material insufficient : 2

related lesions or presence of maternal HPV infection during pregnancy. Babies delivered by vaginal route have greater chances of acquiring infection from HPV positive mothers compared to HPV negative mothers.

Discussion

In this study cervical swabs were collected during the third trimester of pregnancy few days prior to delivery when detectable level of HPV DNA has been reported to reach peak value (Rando et al, 1989, Smith et al, 1991). There is a report (Cason et al, 1995) indicating that for investigating HPV DNA infant buccal swabs are more reliable than nasopharyngeal aspirates.

At birth prevalence of HPV infection among infants was 10.37% and transmission rate was 18.42%. The concept of maternal –fetal transmission of HPV infection at birth was first proposed by Sedlacek et al (1989) and supported by detection of HPV infection in nasopharyngeal aspirates (48%). Also several other studies demonstrated perinatal transmission of HPV infection (Frederick et al, 1993; Cason et al,1995; Syrjanen & Puranen, 2000). Smith et al (1995) and Puranen et al (1996) using PCR reported transmission rates of 4% and upto 80% respectively. Pakarian et al (1994) demonstrated 55% vertical transmission rate, from HPV positive mother to their new-born. In a cohort study with a population of high HPV prevalence (74%) Watts et al (1998) did not find PCR positive nasopharyngeal aspirates at birth. This suggests transient prenatal contamination of infants' specimen by maternal HPV did not occur. In a population with low prevalence (5.2%) of HPV infection among mothers, Tenti et al (1999) detected transmission of 30%. In a previous Indian study, by in situ hybridization (Chatterjee et al, 1998) in a small sample size (N=30) with 40% HPV positive mothers transmission to buccal cells of infants was demonstrated as 41.6%. Review of these literatures demonstrated a widely varying rate of vertical transmission from as low as 0% to as high as 80%. Such variation may be due to the varied risk factors for cervical neoplasia in the population screened, different sample collection techniques and sensitivities of the HPV detection methods.We selected broad-spectrum consensus primer (MY09/MY11) to screen the samples initially for HPV DNA. Only samples positive by consensus primers were genotyped by type specific primers. This may be the cause for lower transmission rate in our study since at least one study shows that consensus primer is less sensitive than type specific primer (Cason et al, 1995).

In our study the high rate of HPV infection among neonates after a cesarean delivery suggests that cesarean delivery did not effectively protect against perinatal transmission of HPV. HPV DNA has been previously found in the amniotic fluid (Armbruster-Moraes et al, 1994), in peripheral blood mononuclear cells of pregnant women and in the cord blood specimens of neonates born to HPV positive mothers (Tseng et al, 1992). This suggests that virus can cross the placental barrier and infection might occur in the uterus.

We found multiple HPV infection among mothers and their infants. A previous report (Liaw et al, 2001) found HPV 16 infection to be usually associated with greater risk of acquisition of other HPV types. Though persistence of the concomitant infections was not affected regardless of types. All HPV positive infants born to HPV positive mothers did not carry the maternal HPV types. We also observed HPV infection among babies from HPV negative mothers. These results are consistent with other reports of discordant mother-infants pairs and HPV positive babies from HPV negative mothers (Syrjanen & Puranen, 2000; Smith et al, 1995). This may be due to HPV transmission by transplacental route in advance of delivery or by some other route (horizontal spread

from relatives/friends, infected fomites/clothing) in the intervening period between delivery and testing (Cason et al, 1998). Additionally, there is evidence that HPV can be found in human sperm cells and HPV specific genes are actively transcribed in infected cells (Lai et al, 1996; Lai et al, 1997). Thus virus infected sperm cells can behave as vectors or carriers for the transmission of HPV to fetuses through fertilized egg (Lai et al, 1996).

Puranen et al (1997) found six infants to be HPV positive even when mothers were negative during delivery. These mothers might have been misdiagnosed as being HPV negative. Such misdiagnosis may be owing to the disease pattern (i.e., fluctuation) in the HPV infected mothers. It is well established that women undergoing a follow up study for prolonged periods may prove alternately and irregularly HPV positive and negative at 6 to 12 months intervals (Schneider et al, 1992; Hildesheim et al, 1994). Fluctuations in the presence of HPV DNA and HPV transient infection in young women have also been described by Schneider et al (1992) and Hinchliffe et al (1995). We also found different HPV types in cervical samples before and after delivery of the women. This is in agreement with the similar findings of another study (Hildesheim et al, 1994).

This study showed no association between HPV infection in infants and maternal age, socioeconomic level, number of pregnancies and prevalence of cervical HPV infection of the mothers. A study by Watts et al (1998) also reported identical observations. Moreover viral load has been found to be a determinant for the perinatal HPV transmission but not influencing persistence of the infection (Kaye et al, 1994).

We were able to test HPV positive infants (10 out of 14) at least once during the follow up period of 1 year and all of them had cleared the HPV infection by this time. Thus presence of HPV DNA in buccal samples possibly did not confirm infection but it might just indicate contamination with infected maternal cells as reported by other studies (Tenti et al, 1999).

A high drop out rate among mothers as well as in their babies was observed in this study. This may be due to maternal reluctance to re-attend or shifting to other areas for child care. Had all the mothers and their babies turned up for the follow-up study the results would have altered to some extent.

Though this study could not conclusively (p=0.0548) support the concept of maternal- fetal transmission of HPV infection even when the infection is sub clinical, there is a clear possibility of transplacental infection of an infant.

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