ORIGINAL ARTICLE

A Study of Cervical Intraepithelial Neoplasia in Pregnancy

**Khanuja Esha · Ghosh U. K. ·
Garg Parul · Tomar Geetika ·
Madan Molly · Bansal Rani**

Abstract

Objective   To find the incidence of human papillomavirus (HPV) infection and cervical intraepithelial neoplasia (CIN) in pregnant women and compare Pap smear with the HPV DNA test in detecting HPV infection.

Materials and Methods   Hundred antenatal women, irrespective of gestational age, were enrolled as subjects in this prospective pilot study for blood investigations, wet mount examination of cervical discharge, Pap smear, and high-risk HPV DNA detection of cervical scrape by PCR. Women showing abnormality in Pap smear and/or those who were high-risk HPV DNA positive were subjected to colposcopy.

Results   The incidence of HPV-positive pregnant women was 18 %. Koilocytosis on Pap smear was observed in six women. Three high-risk HPV DNA-positive women showed changes consistent with CIN 1 on colposcopy.

Conclusions   The HPV DNA test is the most sensitive and reliable in detecting HPV infection as compared to Pap smear, but considering the cost of PCR, Pap smear screening of all antenatal women was recommended.

Keywords   Cervical intraepithelial neoplasia ·
Human papillomavirus · HPV DNA · Pap smear ·
Colposcopy

Khanuja E. · Ghosh U. K. (✉) · Garg P. · Tomar G. ·
Madan M. · Bansal R.
Subharti Medical College, Meerut, India
e-mail: ukghosh@gmail.com

Introduction

Cancer cervix ranks number one among all cancers in women in India over the past two decades and accounted for 16 % of all cancers in women in the urban registries in 2005. The distribution is bimodal, with peaks at 35–39 and 60–64 years of age [1]. It is considered a preventable disease because it has a long preinvasive state [2]. The initiating event in cervical dysplasia and carcinogenesis is infection with HPV, which has been detected in up to 99 % of women with squamous cervical carcinoma [3]. Pregnancy seems to be a risk factor for cervical HPV infection or increased replication of the persisting virus due to the associated increased hormonal level or immunosuppression [4]. Two of the high-risk subtypes 16 and 18 are found in up to 62 % of cervical carcinomas and primarily target basal cells in the stratified squamous epithelium and metaplastic cells within the squamocolumnar junction [5], by interaction of viral E6 and E7 proteins with tumor-suppressor genes p53 and Rb, respectively. A majority of infections are acquired in the first few years after sexual debut and steadily decline thereafter as a result of spontaneous clearance of prevalent infections [6].

The Pap test is the foundation of cervical cancer screening. HPV DNA testing in cervical cancer screening has been the subject of extreme research over the past decade, and when compared with cytology alone, HPV DNA testing is more sensitive, less specific, and has a higher negative predictive value. The combined cytology

and HPV DNA testing has the highest sensitivity, lowest specificity, and the highest negative predictive values [7]. The antenatal period is one event when, under the RCH (Reproductive and Chid Health) program, we have been able to provide at least three checkups to most of our pregnant population, and with this idea, the present study was undertaken to screen antenatal women for preinvasive lesions of the cervix.

Materials and Methods

Hundred consecutive antenatal women, irrespective of parity and gestational age, attending the outpatient department, were taken as subjects with the exclusion criteria of carcinoma cervix, unexplained vaginal bleeding, established labor, and premature rupture of membranes. Only those subjects who had history of at least 7 days of abstinence were considered for the study and investigations. Papanicoloau smear and wet mount (two slides, one with normal saline and other with 10 % KOH) were conducted and simultaneously HPV DNA detection was done from a cervical scrape by Polymerase Chain Reaction using AmpleiGenei HPV Detection Kit supplied by GENEI Bangalore, India, which detected oncogenic HPV types 16, 18, 31, 33, 35, 45, 52b, and 58. Wet mount slides were first examined under a low power and then a high power microscope and any abnormalities (yeast, trichomoniasis, bacterial vaginosis—Amsel's criteria) were noted. Subjects showing evidence of atypical squamous cells of undetermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL), or high grade squamous intraepithelial lesion (HSIL) on Pap smear according to Bethesda classification 2001 [8], or found to be positive for HPV 16 or 18 strains were subjected to video colposcopy under 16× magnification. In this study, HPV-negative cases were considerably more than HPV-positive women. To test the statistical significance of HPV-positive women, the two groups were balanced, i.e., the number of HPV-negative women was made equal to the number of HPV-positive women by random sampling and subsequent statistical testing.

Results

A total of two hundred and eighty-five investigations were done. Out of 100 women, the Pap smears of 99 women were reported with adequate samples, with six of them showing koilocytic changes and high-risk HPV DNA positive as well. None of the HPV-negative women showed koilocytic changes on Pap smear. High-risk HPV DNA by PCR was conducted on all 100 women and the incidence of

HPV-positive women was 18 %. A majority, i.e., 88.88 % (16/18) of HPV-positive cases, were in the age group of 21–30 years (Table 1). The difference in the number of HPV-positive women enrolled in the first, second, and third trimester was not found to be statistically significant ($p > 0.05$) and only four women in this study had their first child birth before the age of 16 years; but, all the women were HPV DNA negative and had inflammatory smear (Table 1). The mean age of marriage of the women in this study was 21.5 ± 3.32 years, with a range of 12–32 years.

The present study showed a positive association of HPV infection with tobacco addiction ($p < 0.05$), but failed to show a significant association with early age at first sexual encounter ($p > 0.05$), the mean age at first sexual encounter of HPV-positive women being 19.9 ± 2.78 years.

Eighty percent of couples in the study were using some form of contraception for spacing, and barrier contraception was practiced by 33 % couples. However, 3/33 (9 %) using the barrier method of contraception were also HPV positive. 72.22 % (13/18) HPV-positive women were practicing some form of contraception, out of which nearly 39 % (7/18) were using oral contraceptive pills (Table 2).

Eighty-one women had vaginal discharge, of which fourteen were HPV positive, while four HPV-positive women had no vaginal discharge (Table 3).

Table 1 Showing distribution of HPV-positive cases according to age, gravidity, gestational age, and age at first child birth

Age (years)	Number of HPV-positive cases ($n = 18$)	Percentage
16–20	1	5.56
21–25	14	77.77
26–30	2	11.11
31 onwards	1	5.56

	Number of HPV-positive cases ($n = 18$)
Gravidity	
G1	6
G2	5
G3	3
G4	1
G5+	3
Gestational age	
First trimester	2/8 (25 %)
Second trimester	7/43 (16.27 %)
Third trimester	9/49 (18.36 %)

Age at first child birth	HPV negative ($n = 82$)	HPV positive ($n = 18$)
Before 16 years	4	0
After 16 years	78	18

Table 2 Showing distribution according to contraceptive use by couple before pregnancy

Type of contraception	Number of cases	
	HPV negative	HPV positive
Barrier	30	3
OCP's	11	7
IUD	9	2
Calendar	10	1
Injectable contraception	7	–
No contraception	15	5
Total	82	18

Table 3 Showing correlation of result of wet mount examination with HPV-negative and HPV-positive cases

Wet smear	HPV negative (67)	HPV positive (14)
Trichomoniasis	2	2
Candidiasis	1	3
Bacterial vaginosis	3	1
Normal	61	8

43 % (6/14) of the HPV-positive women showed co-infection with trichomoniasis, candidiasis, or bacterial vaginosis, whereas only 9 % (6/67) of the HPV-negative women showed evidence of vaginitis and the difference was statistically significant ($p < 0.05$; Table 3).

90.1 % (73/81) of the HPV-negative women and 83.33 % (15/18) of the HPV-positive women showed evidence of various inflammatory changes in Pap smear examination. Two HPV-positive women had normal Pap smear, while one smear had inadequate sample for evaluation.

Discussion

The incidence of HPV DNA-positive pregnant women in this study was 18 %. It varied between 28 and 29 % in other studies [9, 10], probably owing to different geographical areas, religious beliefs, social customs, and the promiscuity rate. 16/18 (88.88 %) HPV-positive women were in the age group of 21–30 years (Table 1), thus showing maximum incidence of HPV infection in the second and third decade of life and a decrease with increasing age, similar to other studies by Worda et al. [11] and Bandyopadhyay and Chatterjee [4].

This study showed that HPV infection was more common in women whose pregnancy order was three or less (14/18, i.e., 77.77 %; Table 1); however, it was difficult to state whether this reflected the actual incidence of HPV infection in gravid women. Therefore, a larger study would be required to elucidate this point. Bandyopadhyay and Chatterjee [4] and Sierra-Torres et al. [12] observed an increasing incidence of HPV with increasing parity.

All 18 HPV-positive women had their first child after the age of 16 years (Table 1), which was similar to study of Vaccarella et al. [13]. Since 18 years is the legal age of marriage of girls in our country, but keeping the prevalent social customs in mind, a cutoff age of 16 years was arbitrarily fixed in this study; it was seen that 95 % of the HPV-negative women and 100 % of HPV-positive women experienced their first child birth after 16 years, showing that this study failed to find an association of first child birth on or before the age of 16 years and HPV infection.

This study showed a positive effect of tobacco use on HPV infection, similar to Ward et al. [14] and contrary to Porras et al. [15]. The present study in contrast to studies by Bandyopadhyay and Chatterjee [4] did not find an increasing incidence of HPV with increasing gestational age, and the possible reason for our observation was the practice of abstinence during pregnancy in our society.

First sexual encounter after the age of nineteen was not a risk factor for HPV infection in this study, which was in agreement with the study by Thomas et al. [16].

Thomas et al. [16] showed oral contraception use being associated with increased risk of CIN grade III among HPV-infected women; yet, we could not conclude with certainty the association of oral contraceptive use with HPV infection because of the small number of subjects in the present study and a lack of data for the duration of use by these women. The observation of 3/18 (16.7 %) HPV-positive women using barrier contraceptive was paradoxical (Table 2), indicating that these couples were probably typical users.

The association of vaginal co-infection with HPV-infected women was found to be statistically significant ($p < 0.05$; Table 3), probably because of the lowered local immunity of the vagina in HPV-infected women. No comparative data were found in the literature on this aspect.

Vaginal infection by wet smear was found in 12 %, whereas by Pap smear in 6 %, and similar results of under-diagnosis by Pap smear were found in studies by Silva Filho [17].

Only six HPV-positive women showed koilocytic changes, which showed that these were in their productive viral infection stage and the rest of the nine were in their latent stage. The women in the productive viral infection stage were advised barrier contraception in addition to six-monthly screening by HPV DNA test and Pap smear, which should be the routine. A similar routine should be observed in women showing latent stage of infection till

both groups show no evidence of HPV infection either by PCR or Pap smear examination.

Conclusions

The incidence of HPV DNA-positive pregnant women in this area of Western Uttar Pradesh (Meerut) was 18 %. Out of the five HPV DNA-positive women, subjected to colposcopy, only three showed changes consistent with CIN 1 and have been counseled for follow-up. HPV DNA testing was found to be the most sensitive and reliable in detecting HPV-infected persons. Pap smear did not show the presence of HPV infection until woman reached the infective stage. Considering the cost of PCR, we recommend routine Pap smear testing for all antenatal women, which would help identify women in the productive viral infection stage.

References

1. Jemal A, Tiwari RC, Murray T, et al. Cervical and vaginal cancer. Berek and Novac's gynecology. 14th ed. Philadelphia: Wolters Kluwer Publishers; 2009.
2. Bidus MA, Elkas JC, Scott Rose G, et al. Cervical and vaginal cancer. Berek and Novac's gynecology. 14th ed. Philadelphia: Wolters Kluwer Publishers; 2009.
3. Walboomers JM, Jacobs MV, Manos MM, et al. Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol. 1999;189:12–9.
4. Bandyopadhyay S, Chatterjee R. HPV viral load determination during pregnancy as a possible cervical cancer risk. J Exp Clin Cancer Res. 2006;25(1):29–38.
5. Wheeler CM. Natural history of human papillomavirus infections, cytologic and histologic abnormalities, and cancer. Obstet Gynecol Clin North Am. 2008;35:519–36.
6. Herrero R, Hildeshiem A, Bratti C, et al. Population based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. J Natl Cancer Inst. 2000;92(6):464–74.
7. Lorinez AT, Richart RM. Human papillomavirus DNA testing as an adjunct to cytology in cervical screening programmes. Arch Pathol Lab Med. 2003;127:959–68.
8. Solomon D, Davey D, Kurman R, et al. The 2001 Bethesda system: terminology for reporting results of cervical cytology. JAMA. 2002;287:2114–9.
9. Bandyopadhyay S, Sen S, Majumdar L, et al. Human papillomavirus infection among Indian mothers and their infants. Asian Pac J Cancer Prev. 2003;4(3):179–84.
10. Aydin Y, Atis A, Tutuman T, et al. Prevalence of human papillomavirus infection in pregnant Turkish women compared with non-pregnant women. Eur J Gynaecol Oncol. 2010;31(1):72–4.
11. Worda C, Huber A, Hudelist G, et al. Prevalence of cervical and intrauterine human papillomavirus infection in the third trimester in asymptomatic women. J Soc Gynecol Investig. 2005;12(6):440–4.
12. Sierra-Torres CH, Acosta-Aragón MP, Orejuela-Aristizabal L, et al. Papillomavirus and factors associated with high-risk, cervical intraepithelial neoplasia in Cauca, Colombia. Ren Salud Publica (Bogota). 2006;1:47–58.
13. Vaccarella S, Herrero R, Dai M, et al. Reproductive factors, oral contraceptive use, and human papillomavirus infection: pooled analysis of the IARC HPV prevalence surveys. Cancer Epidemiol Biomarkers Prev. 2006;15:2148.
14. Ward KK, Berenson AB, Breitkopf CR, et al. Passive smoke exposure and abnormal cervical cytology in a predominantly Hispanic population. Am J Obstet Gynecol. 2011;204:213.
15. Porras C, Bennett C, Safaeian M, et al. Determinants of seropositivity among HPV-16/18 DNA positive young women. BMC Infect Dis. 2010;10:238.
16. Thomas DB, Ray RM, Koetsawang A, et al. Human papillomaviruses and cervical cancer in Bangkok. I. Risk factors for invasive cervical carcinomas with human papillomavirus types 16 and 18 DNA. Am J Epidemiol. 2001;153(8):723–31.
17. da Silva Filho AR. Fresh wet mount in pregnancy: correlation with Pap smears. Rev Bras Ginecol Obstet. 2004;26(7):509–15.