THE JOURNAL OF OBSTETRICS AND GYNECOLOGY OF INDIA

The Journal of Obstetrics and Gynecology of India (July–August 2014) 64(4):279–283
DOI 10.1007/s13224-014-0508-5

ORIGINAL ARTICLE

Prevalence and Clinical Utility of Human Papilloma Virus Genotyping in Patients with Cervical Lesions

Kaur Parminder · Aggarwal Aruna · Nagpal Madhu · Oberoi Loveena · Sharma Swati

Received: 12 December 2012 / Accepted: 21 January 2014 / Published online: 12 April 2014

Abstract

Objective Cervical cancer is the commonest cancer among Indian women. High-risk human papilloma virus (HPV) detection holds the potential to be used as a tool to identify women, at risk of subsequent development of cervical cancer. There is a pressing need to identify prevalence of asymptomatic cervical HPV infection in local population. In our study, we explored the prevalence of HPV genotypes and their distribution in women with cervical lesions.

Methods Scrape specimens were obtained from 100 women (study group) with cervical abnormalities. HPV was detected with amplicor HPV tests, and the individual genotypes in these specimens were identified by Hybribio Genoarray test kit. Fifty specimens were also collected from females with healthy cervix (control group). The present study also aimed to determine the status of HPV prevalence and its association with different sociodemographic factors.

Results Out of the total number of 100 samples, 10 (10 %) women tested positive for HPV DNA. Among them, HPV 18 was observed in 6, HPV 16 in 2, HPV 52 and HPV 39 in one each. Fifty specimens collected from patients with healthy cervix were not infected with any of the HPV genotype.

Conclusions Our study generates data of HPV prevalence in patients with cervical lesions visiting tertiary care institute. The data generated will be useful for laying guidelines for mass screening of HPV detection, treatment, and prophylaxis.

Keywords Cervical cancer · Human papilloma virus · Genotyping

Kaur P., Post Graduate Student · Aggarwal A., Professor and Head · Oberoi L., Professor
Department of Microbiology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, India

Kaur P. (✉), Post Graduate Student
H. No. 1658, Phase 7, Mohali 160062, Punjab, India
e-mail: drparmindergill@yahoo.com

Nagpal M., Professor and Head · Sharma S., Assistant Professor
Department of Obstetrics and Gynaecology, Sri Guru Ram Das Institute of Medical Sciences and Research, Amritsar, India

Introduction

Human papilloma virus (HPV) infection has been reported to be frequently occurring sexually transmitted disease [1]. Although most HPV infections regress, persistent HPV infection, however, is strongly associated with the risk of cervical cancer and genital warts [2].

Cervical cancer is the second most common cancer in the women worldwide [3]. India carries one-fourth of the world's burden of cervical cancer [4]. According to the Indian Council of Medical Research (ICMR), the incidence of cervical cancer in India varies from 20 to 35/100,000 women between the age group of 35–64 years, while in developed countries, it is as

low as 1–8/100,000 women. In India, 1,32,000 new cases are reported annually with 74,000 deaths occurring each year; hence, every 7th minute, a woman dies because of cervical cancer. It is expected that figures are expected to double by 2020 if no action is taken. [5].

Several studies have strongly implicated HPV infection as a causative factor in the development of cervical cancer. The prevalence of HPV in the general population is estimated to be between 9 and 13 % worldwide and varies between 1.6 and 25.6 % country wise [6].

HPVs have been divided into more than 200 genotypes based on DNA sequences, approximately 80 of which have been well characterized [7]. Based on the epidemiologic classification of HPV types by Munoz et al. [8], the high-risk types are 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, and 82; probable high-risk types are 26, 53, and 66; and the low-risk types are 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81, and CP6108.

HPV 2 and HPV 4 are associated with common warts of hands, whereas HPV 6, HPV 11, and others are associated with genital warts. Importantly, 15–20 types of HPV cause virtually all cases of cervical cancer.

Women with normal cervical cytology, who are infected with high-risk HPV type, have an approximately 100-fold increased risk of developing cervical cancer compared with uninfected women. Therefore, it has been suggested that high-risk HPV detection might be used as a tool to identify women at high risk of cervical cancer, in addition to pap smears.

Identification of high-risk HPV genotypes may permit selection of those patients who are at increased risk for disease and may therefore provide additional clinical value. An important requirement for this approach is that HPV testing and identification of high-risk HPV types should be highly sensitive and specific [8]. HPV-infected women remain at risk of developing the disease; hence, its screening is indispensable. Therefore, the present study was designed to determine the prevalence of HPV and its genotypes in women with cervical lesions and then to compare it with the prevalence in women with healthy cervix. Furthermore, in terms of conducting HPV vaccination in India, it is important to understand the prevalence of HPV in the general population.

Materials and Methods

The present study was aimed to determine the prevalence of HPV genotypes in women with cervical lesions. Scrape specimens were prospectively collected from 100 women (study group) with cervical abnormalities. HPV was detected with amplicor HPV test, and the individual genotypes in these specimens were identified by Hybribio Genoarray test kit. Fifty specimens were also collected from females with healthy cervix (control group).

Sample Collection

The sample was collected in the gynecology and obstetrics department and was then sent to the microbiology laboratory in a specimen vial provided. The specimen was collected by introducing a bivalve speculum into vagina for complete visualization of os and ectocervix. The extra secretions were removed using cotton swab, and a brush (as was provided with the Hybribio Genoarray test kit) was inserted into the endocervical canal, rotated at 360° in clockwise direction three to five times, and then the brush was drawn out and put in the specimen vial. The specimen was sent to the microbiology laboratory for further examination.

Processing of Samples

Test Procedure

The whole test is divided into 4 major parts as follows:

(A) DNA extraction by cell lysis solution
(B) PCR amplification
(C) Flow-through hybridization
(D) Result interpretation.

The whole test is done using Hybribio HPV kit.

The Hybribio HPV test kit is designed for detection and determination of 21 specific HPV subtypes in cervical specimens. The 21 HPV types are as follows:

High risk	HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
Low risk	HPV 6, 11, 42, 43, 44
Undetermined risk	HPV 53, CP8304 (81).

Principle of the Test

With the use of polymerase chain reaction principle to amplify extracted HPV DNA from cervical samples, DNA amplicons are then hybridized with immobilized specific HPV probe on the HybriMem under the patented "flow-through hybridization" technique. Enzyme immunoassay method is used for color development to obtain results.

Results

1. A total number of 100 specimens were collected from patients with cervical lesions. Of these, 10 (10 %) were infected with HPV genotypes. Fifty specimens were collected from patients with healthy cervix (control group). No HPV infection was detected in all the 50 specimens of the control group (Table 1).

Table 1 HPV positivity in study group and controls

HPV	Cases	Controls	Total
Positive	10 (10 %)	–	10
Negative	90 (90 %)	50 (100 %)	140
Total	100	50	150

Out of 100 study group cases, 10 (10%) were positive and 90 (90%) were negative.

While among 50 controls, all were negative.

When the prevalence in the study group (10 %) was compared with the control group (0 %), the difference was observed to be statistically significant with $P = 0.021$. This showed that the prevalence of HPV was observed to be more in the study group, i.e., in patients with cervical lesions than that of the control group.

$x^2 = 5.357$; df = 1; $P = 0.021$; Significant at 5 %

Table 2 HPV genotypes detected in study group

Genotype	No. of cases	Percentage
16	2	20
18	6	60
39	1	10
52	1	10
Total	10	100.0

Of the 10 positive cases of HPV, HPV 18 was detected in 6 cases (60 %), HPV 16 in 2(20 %), HPV 39 and HPV 52 in 1 (10 %) each

2. Among the 10 (10 %) HPV-positive cases, HPV 18 was observed in 6, HPV 16 in 2, HPV 52 and HPV 39 in 1 each (Table 2).
3. The presence of HPV was seen in relation to different sociodemographic parameters. The different factors taken into consideration are age, parity, place of residence, and education.
4. Among the 10 HPV-positive females, 8 were in the age group between 25 and 40 years, and one female was between 41 and 50 years, and one between 51 and 60 years.
5. There was no statistically significant difference in HPV positivity between rural and urban populations.
6. Similarly, in the present study, parity in females did not have any association with HPV positivity.
7. Although education contributes to knowing the preventive measures against infectious diseases, in the present study, literacy status was also not related to HPV infection.

Discussion

HPV is recognized as a public health problem for its role as a critical factor in pathogenesis of various cancers. Cervical cancer is a preventable disease [9]. It develops following progression of uncleared HPV infection to high-grade and eventually to invasive disease [10]. Women with normal cervical cytology, who are infected with high-risk HPV, have an approximately 100-fold increased risk of developing CIN 3, compared with uninfected women [11]. Persistence of oncogenic HPV appears essential for the development of cervical neoplasia [10].

The conventional Pap smear has restricted value in identifying women destined to develop cervical neoplasia [12]. The cytologic features of HPV on Pap smear are non-specific. [13].

With the advent of molecular techniques, particularly PCR, it is possible to detect very low quantities of HPV and to subtype the commonly occurring HPV in cervical scrape smears.

In the present study, we evaluated the prevalence of HPV in patients with cervical lesions. Our results showed that the prevalence of HPV in women with cervical lesions was 10 %, whereas in the control group with no cervical lesions, no HPV was detected. The HPV prevalence is in accordance with the other populations studied in India by Bhatla et al. [14] and Kerkar et al. [15].

In a study reported by Bhatla et al. (2008), the prevalence rates of HPV infection among women with normal, low-grade cervical neoplasia (CIN 1), and high-grade CIN (>CIN2) were found to be 7.6, 42.3, and 87.5 %, respectively [14].

The data available from other countries show HPV infection to be 26.8 % in the USA [16]. This can plausibly be attributed to the fact that previous researchers have targeted women in the high-risk groups, viz., women from the rural background or those from the low socioeconomic background.

In India, HPV type 16 alone in cervical cancer is 70–90 %, while the occurrence of HPV type 18 varies from 3 to 20 %. Other high-risk HPV types such as HPV 45, 33, 35, 52, 58, 59, and 73 that have also been reported are rare and thus constitute only a minor group [17–19].

Several studies from India reported that HPV 16 and 18 are the most prevalent HPV types found in India, and the present vaccine against HPV 16 and 18 holds great promise in preventing the women from HPV infection and subsequent development of cervical cancer. However, our study demonstrated HPV type 18 as the most prevalent, followed by HPV 16, HPV 39, and HPV 52. The prevalence of HPV 16/18 in the index study is 8 % which is in accordance with Duttagupta et al. [20] and Aggarwal et al. [21].

Types of HPV in primary screening depend on the population being screened, because of the differences in prevalence of HPV types [21]. Clifford et al. [6] have suggested that cost-effective test could include subset of high-risk HPV, which is most likely to progress to cancer.

Probable incrementing factor for the high prevalence of HPV DNA is of poor hygiene. It is corroborated by the observation that in the index study, of the 10 women positive for HPV, 8 women had chronic cervicitis. Poor hygiene was noted to be associated with a higher prevalence of HPV in women in the control group by Aggarwal et al. [21] as well. In this study, we further tried to determine the association of different sociodemographic factors with HPV infection.

No statistically significant difference was seen among HPV prevalence in rural or urban women. However, in a study by Aggarwal et al. [21], high-risk HPV was more common in rural than the urban women, and the difference was statistically significant ($P = 0.001$). We also found that women who had education status up to 10, +2, or graduation had a significantly higher rate of high-risk HPV.

In many studies, young age [22, 23] was found to be associated with HPV infection, but our study demonstrated no significant association with this factor as was observed by other studies [21]. Our study observed no statistical significant relation of HPV with parity. Duttagupta et al. [20] and Lazcano et al. [24] too did not observe any significant association of HPV with parity.

Cervical cancer screening practices are inconsistent in India. Use of Pap smear, as a sole indicator for screening, has limitations. The cytological interpretation becomes faulty if the smear is inflammatory: a situation not infrequent among women from the low socioeconomic background. In case of infrequent screening, screening with a test of high sensitivity provides greater reassurance that potential disease has not been missed in women who were negative. High-risk HPV DNA screening appears to be a valid option in mass cervical screening programs in developed countries. In a resource-poor country, it is not feasible to offer universal molecular testing for high-risk HPV, till HPV screening is made cheaper. Identification of population at risk will enable focused screening, with a greater cost-effective utilization of resources.

Index study has identified women with chronic cervical lesions to be at a greater risk for HPV compared to the women with healthy cervix. Screening can preferentially be directed to the target population for optimal utilization of resources.

The HPV vaccine can be an important tool for the prevention of cervical cancer. Timely administration before sexual debut will allow control of at least half the cancers. The optimum method of screening for the remainder continues to pose a challenge in India. The rapid HPV test that will be provided at an affordable price to developing countries holds tremendous promise. It can be used as a primary screening method to select patients at risk of disease and allow limited resources to be targeted where they are most needed. A combination of HPV vaccination and HPV-based screening may help control cervical cancer in India.

Conclusions

High-risk HPV DNA screening appears to be a valid option in mass cervical screening programs in developed countries. In a resource-poor country, it is not feasible to offer universal molecular testing for high-risk HPV, till HPV screening is made cheaper. Identification of population at risk will enable focused screening, with a greater cost-effective utilization of resources. Index study has identified women with chronic cervical lesions to be at a greater risk for HPV compared to the women with healthy cervix. Screening can preferentially be directed to the target population for optimal utilization of resources.

The HPV vaccine can be an important tool for prevention of cervical cancer. Timely administration before sexual debut will allow control of at least half the cancers. The optimum method of screening for the remainder continues to pose a challenge in India.

The rapid HPV test that will be provided at an affordable price to developing countries holds tremendous promise. It can be used as a primary screening method to select patients at risk of disease and allow limited resources to be targeted where they are most needed. A combination of HPV vaccination and HPV-based screening may help control cervical cancer in India.

Our study generates data of HPV prevalence with cervical lesions in patients visiting tertiary care institute. The data generated will be useful for laying guidelines for mass screening of HPV detection, treatment, and prophylaxis. The data so obtained may also be relevant in the era of HPV vaccine.

References

1. Burd EM. Human papilloma virus and cervical cancer. Clin Microbiol Rev. 2003; 16:1–17.
2. Braaten KP,Laufer MR. Human papilloma virus (HPV), HPV related disease, and the HPV vaccine. Rev Obstet Gynecol. 2008; 1(1):02–10.
3. Shanta V, Krishnamurthi S, Gajalakshmi CK, et al. Epidemiology of cancer of the cervix: global and national perspective. J Indian Med Assoc. 2000; 98:49–52
4. Speich N, Schmitt C, Bollmann R, et al. Human papillomavirus (HPV) study of 2916 cytological samples by PCR and DNA sequencing: genotype spectrum of patients from the west German area. J Med Microbiol. 2004; 53:125–28
5. Ferlay J, Parkin DM, Pisani P. GLOBOLAN 2002: Cancer incidence, mortality, and prevalence worldwide version 1.0 IARC Cancer Base No. 5. Lyon: IARC press; 2005.

6. Clifford GM, Gallus S, Herrero R, et al. Worldwide distribution of human papillomavirus types in cytologically normal women in the international agency for research on cancer HPV prevalence surveys: a pooled analysis. Lancet. 2005; 366:991–8.
7. Forbes BA, Sahm DF, Weissfeld AS. Laboratory methods in basic virology. Bailey and Scott's diagnostic microbiology. 12th ed. St. Louis: Mosby Elsevier; 2007. p. 729–30.
8. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papilloma virus types associated with cervical cancer. N Engl J Med. 2003; 348: 518–27.
9. Jastreboff AM, Cymet T. Role of the human papilloma virus in the development of cervical intraepithelial neoplasia and malignancy. Postgrad Med J. 2002; 78:225–8.
10. Stanley M. Genital human papillomavirus infections and prospective therapies. J Natl Cancer Inst. 2003; 31:117–24.
11. Rozendaal L, Walboomers JM, van der Linden JC, et al. PCR-based high-risk HPV test in cervical cancer screening gives objective risk assessment of women with cytomorphologically normal cervical smears. Int J Cancer. 1996; 68:766–9.
12. Sherman ME, Lorincz AT, Scott DR, et al. Baseline cytology human papillomavirus testing and risk for cervical neoplasia: A 10 -year cohort analysis. J Natl Cancer Inst. 2003; 95:46–52.
13. Walker JL, Wang SS, Schiffman M, et al. ASCUS LSIL Triage Study Group. Predicting absolute risk of CIN3 during post-colposcopic follow-up: results from the ASCUS-LSIL Triage Study (ALTS). Am J Obstet Gynecol. 2006; 195:341–8.
14. Bhatla N, Dar L, Patro AR, et al. Human papillomavirus type distribution in cervical cancer in Delhi, India. Int J Gynecol Pathol. 2006; 25:398-402.
15. Kerkar SC, Latta S, Salvi V, et al. Human papillomavirus infection in asymptomatic population. Sex Reprod Health. 2011; 2:7–11.
16. Sowjanya AP, Jain M, Poli UR, et al. Prevalence and distribution of high-risk human papilloma virus (HPV) types in invasive squamous cell carcinoma of the cervix and in normal women in Andhra Pradesh, India. BMC Infect Dis. 2005; 5:116.
17. Franceschi S, Rajkumar T, Vaccarella S, et al. Human papillomavirus and risk factors for cervical cancer in Chennai, India: A case-control study. Int J Cancer. 2003; 107:127–33.
18. Kim SJ. Role of colposcopy and cervicography in screening and management of precancerous lesions and early invasive cancer of uterine cervix. J Obstet Gynecol. India 2000;50:134–46.
19. Peedicayil A, Abraham P, Sathish N, et al. Human papillomavirus genotypes associated with cervical neoplasia in India. Int J Gynecol Cancer 2006;16:16(4):1591–5.
20. Duttagupta C, Sengupta S, Roy M, et al. Are Muslim women less susceptible to oncogenic human papillomavirus infection.A study from rural eastern India. Int J Gynecol Cancer. 2004;14:293–303.
21. Aggarwal R, Gupta S, Nijhawan R, et al. Prevalence of high-risk human papillomavirus infections in women with benign cervical cytology: a hospital based study from North India. Indian J. Cancer. 2006;43:110–16.
22. Verteramo R, Pierangeli A, Calzolari E, et al. Direct sequencing of HPV DNA detected in gynaecologic outpatients in Rome, Italy. Microbes Infect. 2006;8: 2517–21.
23. Datta P, Bhatla N, Dar L, et al. Prevalence of human papillomavirus infection among young women in North India. Cancer Epidemiol. 2010;34:157–61.
24. Lazcano-Ponce E, Herrero R, Munoz N, et al. Epidemiology of HPV infection among Mexican women with normal cervical cytology. Int J Cancer. 2001; 91:412–20.