Print ISSN: 2321-6379
Online ISSN: 2321-595X
DOI: 10.17354/ijss/2016/496

Original Article

Screening of High-Risk Women for Human Papillomavirus DNA with Qualitative Polymerase Chain Reaction and its Correlation with Liquid Pap Smear Cytology in a Semi-Urban Population

S Lalitha[1], U Insuvai[2], Heber Anandan[3]

[1]Associate Professor, Department of Radiotherapy, Thoothukudi Government Medical College, Tamil Nadu, India, [2]Senior Resident, Department of Radiotherapy, Thoothukudi Government Medical College, Tamil Nadu, India, [3]Senior Clinical Scientist, Department of Clinical Research, Dr. Agarwal's Healthcare Limited, Tamil Nadu, India

Abstract

Introduction: Cervical cancer is the second most common cancer in women. Human papillomavirus (HPV) is proved by various studies as the common etiologic agent for cervical cancer. So by diagnosing infection with HPV, prevention of developing an invasive cervical cancer is possible.

Aim: To study cervical cancer, screening/secondary prevention is to prevent invasive cervical cancer from developing by detecting and treating women with cervical intraepithelial neoplasia 2/3 lesions.

Materials and Methods: Using reflex panel liquid-based cytology, Pap smear and qualitative HPV L-1 gene was done gel-based polymerase chain reaction (PCR) method for all women in the age group of 25-65 years who attended our OPD with symptoms of leukorrhea.

Results: Qualitative PCR of the vaginal smear samples for HPV DNA testing does not correlate with liquid Pap smear cytology in clinically symptomatic patients with chronic cervicitis.

Conclusion: Cervical cancer screening through HPV DNA qualitative testing by PCR method in women is not reliable and real-time PCR testing to stratify the high-risk types of HPV may be a more accurate method.

Key words: Cervical cancer, Human papillomavirus, Liquid base Pap smear cytology

INTRODUCTION

Cancer of the cervix is the most common gynecological malignancy worldwide.[1] More than 85% of the global burden occurs in developing countries, where it accounts for 13% of all female cancers. Furthermore, the mortality due to cervical cancer is higher in the developing countries where screening and treatment modalities are not commonly available or accessible compared with the developed countries.[2] Every year in India, 122,844 women are diagnosed with cervical cancer and 67,477 dies from the disease. India has a population of 432.2 million women aged 15 years and older who are at risk of developing cervical cancer. It is ranked second most common cancer in women aged 15-44 years. India also has the highest age standardized incidence of cervical cancer in South Asia at 22, compared to 19.2 in Bangladesh, 13 in Sri Lanka, and 2.8 in Iran.[3] Hence, cervical cancer screening should begin at age 21 years. Pap cytology screening is recommended every 3 years for women between the ages of 21 years and 29 years. For women aged 30-65 years, co-testing with cervical cytology screening and HPV testing is preferred and should be performed every 5 years. Cervical carcinoma has its origins at the squamous-columnar junction and it can involve the outer squamous cells, the inner glandular

Access this article online

www.ijss-sn.com

Month of Submission : 07-2016
Month of Peer Review : 08-2016
Month of Acceptance : 09-2016
Month of Publishing : 09-2016

Corresponding Author: Heber Anandan, Dr. Agarwal's Healthcare Limited, No. 10, South Bypass Road, Vannarpettai, Tirunelveli - 627 003, Tamil Nadu, India. Mobile: +91-9894067910. E-mail: clinicalresearch@dragarwal.com

cells, or both. A precursor lesion is dysplasia: Cervical intraepithelial neoplasia (CIN) or adenocarcinoma *in situ*, which can, subsequently, become invasive cancer. This process of tumorigenesis is slow. Longitudinal studies have shown that in 30-70% of patients with untreated *in situ* cervical cancer and will develop invasive carcinoma over a period of 10-12 years.[4] However, in about 10% of patients, precancerous lesions can progress from *in situ* to invasive in a period of <1 year. As it becomes invasive, the tumor breaks through the basement membrane invading the cervical stroma. Extension of the tumor in the cervix may ultimately present as ulceration, exophytic tumor, or extensive infiltration of underlying tissue, including the bladder or rectum. Human papillomavirus (HPV) has been found to be a necessary but not sufficient cause of cervical cancer. Of the more than 100 HPV types, 18 subtypes have been categorized as high-risk types, while the rest are low-risk types for cervical cancer. HPV prevalence among cervical cancer patients in India has varied from 87.8% to 96.67%.[5-7] Molecular studies have shown that HPV-16 and 18 are the two most common highly oncogenic types found in invasive cervical cancer, and out of these two, HPV-16 has been found more commonly.[8] The prevalence of other high-risk types is very low. Hospital-based studies showed a prevalence ranging from 9.9% to 16.6% among women with benign cervical cytology.[9,10] The critical components of a screening program are an acceptable good-quality screening test, prompt diagnostic investigations, appropriate treatment, and post-treatment follow-up. There is a strong evidence from non-experimental studies in developed countries such as Denmark and Finland that the incidence and mortality of cervical cancer can be reduced by screening.[11]

Aim

To study cervical cancer screening/secondary prevention is to prevent invasive cervical cancer from developing by detecting and treating women with CIN2/3 lesions.

MATERIALS AND METHODS

Location of the study conducted: Cancer OP, Thoothukudi Government Medical College, Thoothukudi.

Using reflex panel liquid-based cytology, Pap smear and qualitative HPV L-1 gene was done gel-based polymerase chain reaction (PCR) method for all women in the age group of 25-65 years who attended our OPD with symptoms of leukorrhea. The interpretation was done by the presence or absence of 158 base pair product of HPV DNA. The sensitivity and specificity of HPV testing were compared with routine cytology, both overall and for various age groups.

RESULTS

Around 46% (10/22) of the screened population was in the 41-50 age groups. This age group confirms to that of the active sexual life and reproductive age (Table 1).

Abnormal findings were seen on clinical examination in 55% (12/22) of the screened population. The findings noted were hypertrophy of cervix (1/22), erosions in anterior, posterior, or both the cervical lips (9/22) (Figure 1).

Liquid Pap smear cytology was done using the reflex panel. It showed evidence of dysplasia in 83% of the screened population. Mild dysplasia was seen in 4% and moderate dysplasia was seen in 13% (Figure 2).

Qualitative HPV L - 1 gene analysis done by gel-based PCR method was negative in all the 22 persons (100%) screened (Figure 3). Screened population with positive clinical findings when compared with the results of liquid Pap smear cytology. Leukocyte particle concentration was positive in only 2/7 persons who had positive clinical findings but was negative in all the 15 persons with negative clinical findings, $P = 0.091$ (Table 2).

Qualitative HPV L - 1 gene PCR was negative in all the persons with both positive and negative clinical findings, $P = 1.000$ (Table 3).

Table 1: Distribution of study patients

Age group	Patients (%)
20-30	4 (18)
31-40	10 (46)
41-50	4 (18)
51-60	4 (18)

Table 2: Cross tabulation of clinical findings with LPC

Cross tab	LPC		*P* value
	Positive	Negative	
Clinical finding			
Positive	2	5	0.091
Negative	0	15	

LPC: Leukocyte particle concentration

Table 3: Cross tabulation of clinical findings with PCR

Cross tab	PCR		*P* value
	Positive	Negative	
Clinical finding			
Positive	0	7	1.000
Negative	0	15	

PCR: Polymerase chain reaction

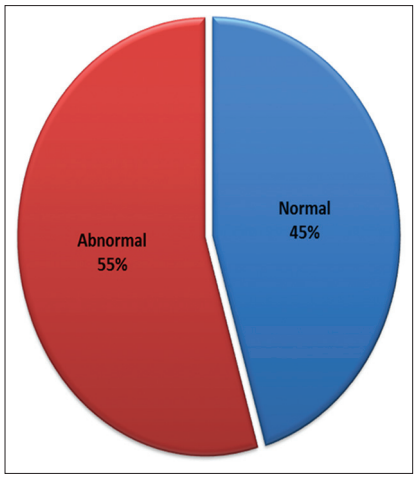

Figure 1: Pervaginal examination findings

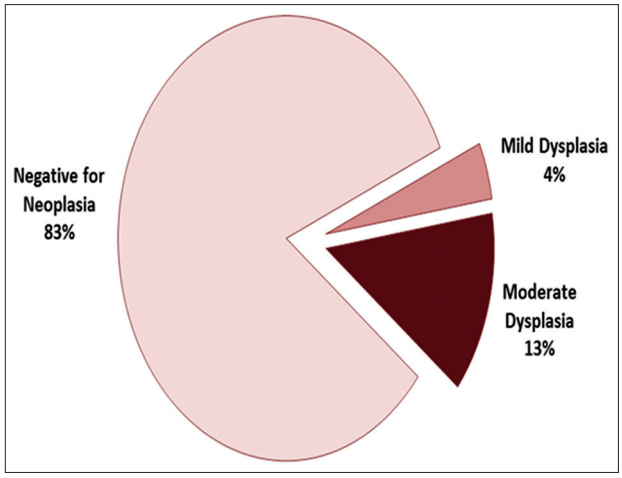

Figure 2: Liquid Pap smear cytology findings

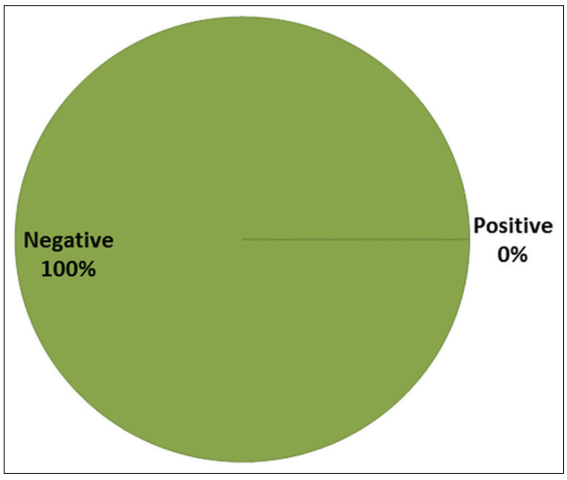

Figure 3: Qualitative human papillomavirus L-1 gene analysis

DISCUSSION

Currently, in Europe and North America, cervical cancer screening is based on exfoliative cytology performed at intervals ranging between 1 and 5 years. There has been a marked reduction in incidence and mortality rates of squamous cell carcinoma of the cervix in countries with established cytology screening programs.[12-14]

Cytology had a higher positive predictive value than HPV testing, which reduces the costs associated with referral for colposcopy. However, in well-screened populations, its lower sensitivity is associated with a high proportion of cancers occurring in apparently adequately screened women.[13]

The intent of this study was, if HPV qualitative analysis showed positivity, with a normal Pap smear, then the tests have to be repeated after 1 year as per the recommendations.[15] If Pap smear was abnormal, all these persons require colposcopy and directed biopsy. If there is cervicitis clinically, with normal Pap smear cytology and negative HPV, the tests should be repeated after 1 year and should be under close follow-up.[16]

However, our study showed that qualitative PCR for HPV DNA has very low sensitivity and specificity in clinically symptomatic individuals and liquid Pap smear cytology also showed low detection of CIN lesions in the sample studied.

CONCLUSION

The results of the above study show that qualitative PCR of the vaginal smear samples for HPV DNA testing does not correlate with liquid Pap smear cytology in clinically symptomatic patients with chronic cervicitis. Hence, quantitative PCR testing is required to stratify the high-risk HPV types.

REFERENCES

1. Sowjanya AP, Jain M, Poli UR, Padma S, Das M, Shah KV, *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.
2. Sideri M, Cristoforoni P, Casadio C, Boveri S, Igidbashian S, Schmitt M, *et al.* Distribution of human papillomavirus genotypes in invasive cervical cancer in Italy: A representative, single institution case series. Vaccine 2009;27 Suppl 1:A30-3.
3. Human Papillomavirus and Related Diseases Report. ICO Information Centre on HPV and Cancer (HPV Information Centre). 2016.
4. Cervical Cancer Treatment. National Cancer Institute. 2016. Available from: http://www.cancer.gov/types/cervical/hp/cervical-treatment-pdq. [Last cited on 2016 Aug 20].
5. Kulkarni SS, Kulkarni SS, Vastrad PP, Kulkarni BB, Markande AR, Kadakol GS, *et al.* Prevalence and distribution of high risk human papillomavirus (HPV) Types 16 and 18 in carcinoma of cervix, saliva of patients with oral squamous cell carcinoma and in the general population in Karnataka, India. Asian Pac J Cancer Prev 2011;12:645-8.
6. Gheit T, Vaccarella S, Schmitt M, Pawlita M, Franceschi S, Sankaranarayanan R, *et al.* Prevalence of human papillomavirus types in

cervical and oral cancers in central India. Vaccine 2009;27:636-9.
7. Basu P, Roychowdhury S, Bafna UD, Chaudhury S, Kothari S, Sekhon R, *et al.* Human papillomavirus genotype distribution in cervical cancer in India: Results from a multi-center study. Asian Pac J Cancer Prev 2009;10:27-34.
8. Bhatla N, Dar L, Rajkumar Patro A, Kumar P, Pati SK, Kriplani A, *et al.* Human papillomavirus-type distribution in women with and without cervical neoplasia in north India. Int J Gynecol Pathol 2008;27:426-30.
9. Srivastava S, Shahi UP, Dibya A, Gupta S, Roy JK. Distribution of HPV genotypes and involvement of risk factors in cervical lesions and invasive cervical cancer: A study in an Indian population. Int J Mol Cell Med 2014;3:61-73.
10. Aggarwal R, Gupta S, Nijhawan R, Suri V, Kaur A, Bhasin V, *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;43:110-6.
11. Hakama M, Chamberlain J, Day NE, Miller AB, Prorok PC. Evaluation of screening programmes for gynaecological cancer. Br J Cancer 1985;52:669-73.
12. Laara E, Day NE, Hakama M. Trends in mortality from cervical cancer in the Nordic countries: Association with organised screening programmes. Lancet 1987;1:1247-9.
13. Sasieni PD, Cuzick J, Lynch-Farmery E. Estimating the efficacy of screening by auditing smear histories of women with and without cervical cancer. the national co-ordinating network for cervical screening working group. Br J Cancer 1996;73:1001-5.
14. IARC Handbook of Cancer Prevention Volume 10 - Cervix Cancer Screening. Iarc.fr. 2005. Available from: http://www.iarc.fr/en/publications/pdfs-online/prev/handbook10/. [Last cited on 2016 Aug 29].
15. The American Congress of Obstetricians and Gynecologists. ACOG Practice Bulletin. Clinical Management Guidelines for Obstetrician-Gynecologists: Screening for Cervical Cancer. November, 2012.
16. Katki HA, Kinney WK, Fetterman B, Lorey T, Poitras NE, Cheung L, *et al.* Cervical cancer risk for women undergoing concurrent testing for human papillomavirus and cervical cytology: A population-based study in routine clinical practice. Lancet Oncol 2011;12:663-72.

How to cite this article: Lalitha S, Insuvai U, Anandan H. Screening of High-Risk Women for Human Papillomavirus DNA with Qualitative Polymerase Chain Reaction and its Correlation with Liquid Pap smear Cytology in a Semi-Urban Population. Int J Sci Stud 2016;4(6):109-112.

Source of Support: Nil, **Conflict of Interest:** None declared.