ORIGINAL RESEARCH PAPER

Volume-7 | Issue-5 | May-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PREVALENCE OF HPV INFECTION AMONG FEMALES VISITING THE GYNECOLOGY OPD OF SHREE KRISHNA HOSPITAL, KARAMSAD.

Pathology

Ms. Soumya Mohanan* P.hd student, Department of Pathology, Pramukh Swami Medical College, Karamsad-388 325. *Corresponding Author

Dr. Menka Shah Professor, Department of Pathology, Pramukh Swami Medical College, Karamsad-388 325.

Mr. Piyush Chudasama Research Scientist, Akanksha Hospital, Lambhvel.

ABSTRACT

Background: Cervical cancer is one of the leading causes of death for middle-aged women in the developing world, yet it is almost completely preventable if precancerous lesions are identified and treated in a timely manner. There are different methods for control and prevention of cervical cancer which include conventional cytology (Pap smear), liquid-based cytology, human papillomavirus (HPV) screening, and vaccination against HPV. It is now well established that HPV infection is the central, probably necessary cause of cervical cancer.

Aim: To study the prevalence of HPV infection among females visiting the Gynecology OPD of Shree Krishna Hospital.

Materials and Methods: Subjects for the proposed study were selected randomly from the female patients attending Gynecology Opd of Shree Krishna Hospital, Karamsad. A total of 115 cervical cytology samples were studied.

Results: In this study, out of 115 patients, 5(4.35%) patients were positive for HPV infection.

Conclusion: Our study generates data of HPV prevalence in patients visiting Gynecology Opd of Shree Krishna Hospital, Karamsad. The data generated will be useful for laying guidelines for mass screening of HPV detection, treatment, and prophylaxis

KEYWORDS

Cervical cancer, Human papilloma virus, liquid-based cytology.

Introduction:

Cervical cancer is one of the leading causes of death for middle-aged women in the developing world, yet it is almost completely preventable if precancerous lesions are identified and treated in a timely manner. There are different methods for control and prevention of cervical cancer which include conventional cytology (Pap smear), liquid-based cytology, human papillomavirus (HPV) screening, and vaccination against HPV.[1]

It is now well established that HPV infection is the central, probably necessary cause of cervical cancer.[2] Statistical analyses released from the World Health Organization (WHO) suggest that cervical cancer is the second most common cancer in women worldwide.[3,4] It is estimated that each year approximately 493,000 new cases are diagnosed and 274,000 women die from cervical cancer worldwide.[5] Human papillomavirus is considered to be the most important risk factor in the development of cervical cancer and sexual transmission is the predominant route of HPV infection. Transitional zone of cervix is the most common site of cervical cancer and it is most susceptible to the carcinogenicity of HPV.[6] There are an estimated 132,000 new cases and 74,000 die of this preventable disease in India each year.[7]

HPV is a double-stranded DNA virus that is non-enveloped and has an icosahedral capsid. The virus replicates as an extra chromosomal DNA inside the nucleus of the host cell.[8] At present, about 118 different types of HPV have been characterized;[9] depending on the risk of malignancy, HPVs are further grouped as high risk or low risk types. The etiopathogenesis of cervical cancer is indeed complex, and the progression to cancer generally takes place over a period of 10 to 30 years. HPV 16 and 18 are considered the most prevalent high risk types for carcinogenesis while HPV types 6 and 11 are the most prevalent low risk types with benign and genital warts.[10,11] Further, there are thirteen more high-risk HPV types viz. 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82, and three probable high risk types viz. 26, 53, 66.[12] Out of these, HPV 16 and 18 are estimated to account for about 70% of all cervical cancers and altogether HPV 16, 18, 45, 31, 33, 35, 52, 58 are responsible for about 90% of all cervical cancers worldwide.[13] In addition to the most prevalent low-risk HPV types HPV 6 and 11, other types are 40, 42, 43, 44, 54, 61, 70, 72 and 81.[14] Cervical cancer is a major public health problem in India. Screening strategies for reducing the burden of HPV-mediated carcinogenesis are emerging as an effective means for cervical cancer control and prevention in developing countries. Organizing screening programs in developing nations is indeed a big challenge. The key to reducing cervical cancer morbidity and mortality is early detection coupled with timely treatment of cervical precancerous lesions.

The purpose of the study is to determine the prevalence of human papillomavirus in the group studied.

Materials & Methods:

Subjects for the proposed study were selected randomly from the patients attending Gynecology Opd of Shree Krishna Hospital, Karamsad. A total of 115 cervical cytology samples taken from the females visiting the Gynecology Opd of Shree Krishna Hospital, Karamsad were studied. Cervical cytology samples from all women from 18-75 years attending the Gynecology Opd of Shree Krishna Hospital, Karamsad were included. The study participants were randomly selected on the basis of complaints like bleeding per vaginum, irregular menses, pain in lower abdomen, post coital bleeding or any abnormal findings on per speculum examination and patients of health checkup who came for cervical screening without any symptoms were also included. Non co-operative patients, Patients who do not give consent, pregnant women, patients with massive bleeding per vagina/ at the time of menstruation were excluded. Informed written consent was taken in the prescribed format from all the participants before collecting samples for the study.

LIQUID BASED CYTOLOGY (THIN-PREP)

The ThinPrep® System: The heart of the ThinPrep System is the ThinPrep 2000 Processor, an automated slide preparation unit that produces remarkably uniform thin-layer slides, finely free of obscuring artifacts such as blood, mucus and inflammation.

Step 1: A gynecologic sample was collected using a broom-type or cytobrush/spatula cervical sampling device.

Step 2: Instead of smearing the cells on a slide, the sampling device was rinsed into a ThinPrep vial containing PreservCyt® transport medium. The device was then discarded.

Step 3: The sample vial was capped, labeled, and taken to the laboratory for slide preparation.

Step 4: The residual material after slide preparation is used for HPV DNA detection.

HPV DNA DETECTION BY POLYMERASE CHAIN REACTION

Isolation of genomic DNA from thin prep collection vial after the preparation of slide was done by using standardized manual method.

Manual Protocol for isolation of genomic DNA from thin-prep vial:-

1. Sample is taken in a 15 ml falcum tube.
2. Centrifuge at 3000 rpm for10 min.
3. Discard supernatant and add 500 µl of 1X TE.
4. Mix the cells & transfer to 2ml tube.
5. Add 300 µl lysis buffer. (3% SDS, 2X TE, pH 8.0)
6. Add 7.5 µl of Proteinase K (d10 mg/ml) to bring a final conc. of 100 µg/ml of PK.
7. Mix carefully 4-5 times. Avoid froth formation.
8. Incubate overnight at 50ºC in a water bath OR at 65 ºC for 3 hours.
9. Add an equal volume (750 µl) of phenol (Tris-equilibrated).
10. Mix & Centrifuge at 7000 rpm for 10 min.
11. Transfer distinct upper aqueous phase to a fresh, sterilized 2 ml tube.
12. Add an equal volume of phenol and CIA (Chloroform:iso-amyl alcohol[24:1].
13. Repeat steps 10 & 11.
14. Add equal volume of chilled isopropanol.
15. Mix gently to precipitate the DNA.
16. Store at -20 ºC for half an hour.
17. Centrifuge at 10000 rpm for 10 min to pellet the DNA.
18. Discard supernatant carefully and perform washing steps using 70% ethanol to remove of traces of salts.
19. Add 500 µl of 70% ethanol.
20. Gently mix twice or thrice.
21. Centrifuge at 10,000 rpm for 10 min.
22. Discard supernatant carefully.
23. Again add 200 µl of 70% ethanol.
24. Repeat steps 20, 21, 22.
25. Add 100 µl of 70% ethanol.
26. Repeat steps 20, 21, 22.
27. Leave the DNA pellet in a 37 ºC sterile incubator for 45-60 min to dry.
    OR
28. Leave O/N at RT in a sterile hood/laminar air flow to dry the DNA pellet.
29. Add appropriate amount of 1X TE depending upon the size of DNA pellet (10 µl to 500 µl).

After the isolation, concentration & quality of genomic DNA was checked by QIAexpert.

Storage of the elute with purified DNA:

The elute contains pure genomic DNA. For short-term storage (24-48 hrs.) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures

POLYMERASE CHAIN REACTION

Polymerase Chain Reaction was done for the detection of house-keeping gene (Actin) as an internal control for all the samples.
Polymerase Chain Reaction was done for the detection of Human papilloma virus.

Protocol of PCR for Actin & HPV:

Following reaction system was prepared in sterile PCR tubes. Here ready to use master mix (Emerald) was used for the PCR.

Reaction system (20µl/ reaction tube) for PCR:

Reagent	Volume per reaction tube
Test DNA	2 µl
Forward Primer	0.5 µl
Reverse Primer	0.5 µl
Master mix	10 µl
Double distilled water	7 µl
Total volume	20 µl

After preparing above reaction system, all the reaction tubes were kept in Veriti 96-well thermal cycler and was allowed to run for the following programme.

Standardized PCR programme for ACTIN:

Stage 1×1 95ºC for 3 minutes

Stage 2 ×35 95ºC for 15 seconds
56ºC for 30 seconds
72ºC for 30 seconds

Stage 3 × 1 72ºC for 5 minutes
4ºC –hold

Standardized PCR programme for HPV:

Stage 1×1 95ºC for 2 minutes

Stage 2 ×35 95ºC for 15 seconds
50ºC for 30 seconds
72ºC for 30 seconds

Stage 3 × 1 72ºC for 5 minutes
4ºC -hold

Previously confirmed HPV positive DNA was used as a positive control for HPV DNA detection. After completion of run in thermocycler, 10µl from each reaction tube was loaded on 2% Agarose gel. After completing the electrophoresis run PCR products were visualized by the gel documentation system.

Results and Discussion:

The present study was carried out on a total of 115 patients visiting the gynecology OPD of Shree Krishna Hospital, Karamsad who were selected randomly at a particular point of time. From 115 patients, two samples were taken, one for conventional Pap smear and the other for liquid based cytology. Residual material from liquid based cytology vial was used for Human Papillomavirus detection. Reporting of smears strictly followed Bethesda system 2001.

Table 7: Prevalence of Human Papillomavirus Infection

HPV BY PCR	FREQUENCY	PERCENTAGE	CUMULATIVE FREQ.
ABSENT	110	95.65	95.65
PRESENT	5	4.35	100.00
TOTAL	115	100.00	

Table-7, shows prevalence of Human Papillomavirus Infection in the group studied. Out of 115 patients, 5(4.35%) patients were positive for HPV infection.

Table 8: Age-wise distribution of Human Papillomavirus infection.

AGE GROUP	HPV DNA by PCR		Total	P-Value
	Absent	Present		
26-35	7 6.36	0 0.00	7 6.09	0.380
36-45	36 32.73	0 0.00	36 31.30	
46-55	27 24.55	2 40.00	29 25.22	
56-65	34 30.91	2 40.00	36 31.30	
66-75	6 5.65	1 20.00	7 6.09	
TOTAL	110 100.00	5 100.00	115 100.00	

Table 8 shows HPV infection in association with age. Highest prevalence 2(40%) is seen in age group 46-55 & 56-65 followed by 1(20%) in age group 66-75. Though the present study reveals no significant association of HPV with age, it showed highest prevalence of HPV infection in >50 years of age group which may be due to persistent HPV infection.[15]

Conclusion:

In this prospective cross sectional study, a total of 115 patients were

studied. Residual material from liquid based cytology vial was used for Human Papillomavirus detection. Out of 115 patients, 5(4.35%) patients were positive for HPV infection. This study showed the prevalence of HPV in >50 years group which may be due to persistent HPV infection. If HPV testing and cytology testing are done in parallel, the high negative predictive value means that women who test negative with both methods receive a very high level of reassurance that they may not be at risk of cervical cancer for a long time.

References:

1. Ahmed Ibrahim; Vibeke Rasch; Eero Pukkala; Arja R Aro. Cervical cancer risk factors and feasibility of visual inspection with acetic acid screening in Sudan. International Journal of Women's Health 2011, 3:117-122.
2. Bosch FX, Lorincz A, Mufioz N, Meijer CJ, Shah KV. The causal relation between human papillomavirus and cervical cancer. J Clin Pathol. 2002;55:244–265.
3. Pisani P, Parkin M, Baray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. Int J Cancer. 1999;83:18–29.
4. Pisani P, Bray F, Parkin DM. Estimates of the world-wide prevalence of cancer for 25 sites in the adult population. Int J Cancer. 2002;97:72–81.
5. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics 2002. CA Cancer J Clin. 2005;55(2):74–108.
6. Yousuf S, Syed S, Moazzam A, Lucky MH. Frequency of high risk human papillomavirus types in squamous cell carcinoma of cervix among women. J Pak Med Assoc. 2010;60(3):193–196.
7. Ferlay J, Bray F, Pisani P, Parkin DM. GLOBOCAN 2002: Cancer incidence, mortality and prevalence worldwide, IARC CancerBase No. 5, Version 2.0, Lyon: International Agency for Research on Cancer Press; 2004.
8. Longworth MS, Laimins LA (2004). Pathogenesis of human papillomaviruses in differentiating epithelia. Microbiol. Mol Biol Rev, 68, 362-72.
9. Jo H, Kim JW (1998). Implications of HPV infection in uterine cervical cancer. Sex Transm Inf, 74, 101-9.
10. Motoyama S, Ladines-Llave CA, Luis Villanueva S, Maruo T (2004). The role of human papillomavirus in the molecular biology of cervical carcinogenesis. Kobe J Med Sci, 50, 9-19.
11. Li TT, Zhao LN, Liu ZG, Han Y, Fan DM (2005). Regulation of apoptosis by the papillomavirus E6 oncogene. World J Gastroenterol, 11, 931-7.
12. Zur Hausen H (1991). Viruses in human cancers. Science, 254, 1167-73.
13. Bosch F (2003). Epidemiology of human papillomavirus infections: new options for cervical cancer prevention. Salud Publica Mex, 45, 326-39.
14. Villers EM, Fauquet C, Broker TR, Bernard HU, Zur Hausen H (2004). Classification of papillomaviruses. Virology, 324, 17-27.