

Original Research Article

High Risk Human Papilloma Virus (HR-HPV) Genotyping in Female with Abnormal Cervix

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Abstract: Human Papilloma Virus (HPV) infection is transmitted primarily via sexual and affects mostly basal epithelial cells of the skin or inner lining of tissues and are categorized as cutaneous types or mucosal types. It is known that HPV infection is strongly related to the development of cervical cancer, as viral DNA is detected in 99% of cervical cancer cases worldwide. Proposed study includes 14 cervical brushing, collected from females with cytologically abnormal cervix. The target genes E6 and E7 were amplified by Conventional PCR. Three cases with High grade squamous intraepithelial lesion (HSIL) came positive. Further the positive cases were digested by restriction endonucleases enzymes for RFLP and the amplicon size for high risk HPV type 16 and type 18 were obtained at 452bp and 455bp respectively. Three cases came positive, out of which HPV type 16 came in two cases with one HPV type 18. High risk HPV characterization at an early stage will prevent further progression of the disease.

Keywords: Oncogenic, Restriction endonucleases, Venereal Diseases, Cutaneous, Abnormal cervix.

INTRODUCTION:

Sexually transmitted infections (STIs), and Venereal Diseases (VD), commonly spread by sexual activities, especially vaginal intercourse, anal sex and oral sex [1]. Most STIs do not cause symptoms. More than 30 different bacteria, viruses, and parasites can cause STIs. Bacterial STIs includes Chlamydia, gonorrhea, and syphilis among others. Each year, there are an estimated 357 million new infections with 1 of 4 STIs: chlamydia, gonorrhoea, syphilis and trichomoniasis [2, 3]. More than 500 million people are estimated to have genital infections with herpes simplex virus (HSV). More than 290 million women have a human papillomavirus (HPV) infection [4-6]. Drug resistance, especially for gonorrhoea, is a major threat to reducing the impact of STIs worldwide. Viral STIs includes genital herpes, Human Immunodeficiency Virus /AIDS and Human Papilloma Virus (HPV) and genital warts among others [7, 8]. HPV is a small, non-enveloped and double stranded DNA virus with a genome of approximately 8,000 nucleotides and with the diameter of 55nm. It establishes infection on basal epithelial cells of the skin or inner lining of tissues and is categorized as cutaneous types or mucosal types.

More than 110 genotypes have been cataloged and sexually transmitted. Thus in the current work Human Papilloma Virus Genotypes were Characterized by Restriction Fragment Length Polymorphism (RFLP) Polymerase Chain Reaction (PCR) in females with Abnormal Cervix.

MATERIALS AND METHODS:

Clinical specimen's mainly cervical brushings were collected from females with abnormal cervix in virus transport medium. Further the DNA was extracted from all the specimens. Selection of the target HPV DNA sequences has been based on the study of highly conserved regions in the HPV genome for tested generic genotypes (GEN1 and GEN2 primers), and of minimally variable regions for tested oncogenic HPV genotypes (ONC1 and ONC2). Selected regions have a high degree of conservation between the tested genotypes. Pair 1 (GEN1 and GEN2) defines a segment of approximately 400 bp, while Pair 2 (ONC1 and ONC2) defines a sequence of approximately 250 bp in oncogenic HPV genotypes. The master mix was prepared for HPV oncogenes, mainly early genes designated as E6 and E7. Amplification was done for

these target genes. Amplification was done by setting the cycling parameters as; initial duration for 94°C for 5 minutes, followed by 35 repetitive cycles of denaturation at 94°C for 30seconds, annealing at 55°C for 1 min, followed by extension at 72°C for 1 min (13,14). Final extension was given at 72°C for 10 minutes. We used 3B Black biotech kit for the amplification of E-6 and E-7 genes of HPV genome. Aliquot 40 µl of the Reaction Mixture in each amplification vial from the kit. Add 50-100ng DNA from the purified samples to each reaction vial. Complete up to 50 µl final volume with sterile distilled water. RFLP Analysis: Amplification products are subjected to a restriction digestion using five different restriction endonucleases. The obtained patterns

indicate the presence of oncogenic HPV genotypes. For RFLP analysis we performed five enzymatic digestions for each amplification. Prepared each Digestion mixture following the indications as: Digestion A: 1 µl Enzyme A + 1 µl Buffer A, Digestion B: 1 µl Enzyme B + 1 µl Buffer B, Digestion C: 1 µl Enzyme C + 1 µl Buffer C, Digestion D: 1 µl Enzyme D + 1 µl Buffer D/E, Digestion E: 1 µl Enzyme E + 1 µl Buffer D/E. Detection of digested products is performed by agarose gel electrophoresis followed by ethidium bromide staining. 3B biotype kit. In agarose gel electrophoresis, prior to RFLP, the presence of HPV is indicated by a band of approximately 450 bp. If oncogenic HPV genotypes are present in the sample, an additional band of 250 bp will also appear.

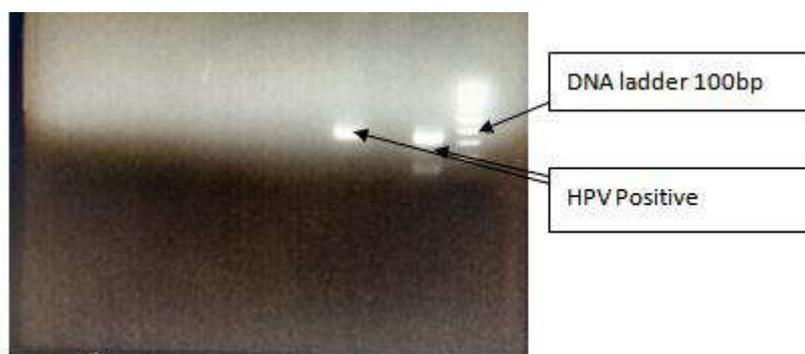


Fig.1: Gel picture depicting the band of oncogene (252 bp amplicon size)
Well 1 and Well 3- HPV Positive
Well 2- HPV Negative
Well 3- DNA Ladder 100bp



Fig 2: Agarose gel electrophoresis RFLP Picture
Well 1- Restriction Enzyme A + Buffer A- Negative
Well 2- Restriction Enzyme B + Buffer B -Negative
Well 3- Restriction Enzyme C + Buffer C -Positive
Well 4- Restriction Enzyme D + Buffer D/E -Negative
Well 5- Restriction Enzyme E + Buffer D/E - Negative
Well 6- DNA Ladder 100bp.

RESULTS

A total of 14 specimens were collected from females with cytologically abnormal cervix. The specimens were collected from Shri Mahant Indresh Hospital which includes Gynecology Department and

further they were processed and their DNA was isolated by silica column method. The target genes E6 and E7 were amplified by PCR. Amplicon size of 252bp was obtained for Oncogenic HPV. Further the positive cases were digested by restriction endonucleases enzymes for

RFLP and the amplicon size for high risk HPV type 16 and type 18 were obtained at 452bp and 455bp respectively. Three cases came positive, out of which HPV type 16 came in two cases with one HPV type 18.

DISCUSSION AND CONCLUSION

HPV infection is transmitted primarily via sexual activity. Half of all new cases are detected during the first three years of sexual activity [9]. Genotyping by PCR-RFLP allows the HPV to be typed, and is easier and less expensive than sequencing. The method is simple and robust, does not require sophisticated equipment, and is particularly suited to settings in which financial resources are limited [10, 11]. PCR-RFLP shows good discriminatory power by differentiating the virus in High Risk or Low Risk, and it is possible to identify single or multiple infections. In this technique, the amplified DNA is digested by restriction enzymes, resulting in DNA fragments of various lengths. Molecular techniques are most commonly used for HPV testing, and are the gold standard for diagnosing the viral infection. Some of these methods may also be used for investigation of regional and country-based type specific HPV prevalence.

Cell infection by HPV is shown by changes in function or in host gene expression, and the detection of these changes may play a major role in the screening and follow-up of infected patients [12]. In spite of their value, molecular techniques still must become more rapid, automated, and low-cost to be of practical use in low-income population and countries. Present study came up with the presence of HPV type 16 in the females with abnormal cervix along with the HPV type 18. The study was limited to the 14 numbers of cases and can be further done for more findings.

Conflict of Interest: None

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REFERENCES:

1. Fleming DT, Wasserheit JN. From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection. Sexually transmitted infections. 1999 Feb 1; 75(1):3-17.
2. Sharma N, Sharma A, Yadav D, Saraswat N, Singh S, Govil RK, Sharma C. Molecular Genetics of Human Papillomavirus-Significant for Cervical Cancer Diagnosis and Management.
3. Taira AV, Neukermans CP, Sanders GD. Evaluating human papillomavirus vaccination programs. Emerg Infect Dis. 2004 Nov 1; 10(11):1915-23.

4. HPV-the Shy Virus" (radio program). Sound print. 6 December 2008. Retrieved 6 December 2008.
5. Sharma N, Kandpal J, Jawed B, Pandita A, Razdan N, Rawat S, Singh A, Sharma B, Deep A, Pawar M. Human Papillomavirus Genotyping by Dual Priming Oligonucleotide Technology And Its Clinical Efficacy in Cervical Cancer Management.
6. World Health Organization, International Agency for Research on Cancer. Human papillomaviruses. World Health Organization; 2007.
7. Dunne EF, Unger ER, Sternberg M, McQuillan G, Swan DC, Patel SS, Markowitz LE. Prevalence of HPV infection among females in the United States. Jama. 2007 Feb 28; 297(8):813-9.
8. Zur Hausen H. FOUNDATIONS IN CANCER RESEARCH. Advances in Cancer Research. 1996 May 9; 68:1.
9. Sinal SH, Woods CR. Human papillomavirus infections of the genital and respiratory tracts in young children. In Seminars in pediatric infectious diseases 2005 Oct 31 (Vol. 16, No. 4, pp. 306-316). WB Saunders.
10. Moscicki AB. Impact of HPV infection in adolescent populations. Journal of Adolescent Health. 2005 Dec 31; 37(6):S3-9.
11. Sharma N, Sharma V, Singh PR, Kushwaha RS, Nautiyal SC, Sailwal S, Singh RK, Masood T, Mishra P, Singh RK. Age wise distribution of high risk Human Papillomavirus in Northern Indian women. Biomed Res. 2012; 23(4):547.