



Original Article

Amplification of Early genes (E6 and E7) in Oncogenic Human Papilloma Virus- Clinical Relevance for Disease Screening

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The Human Papilloma Virus (HPV) is one of the most common causes of sexually transmitted disease in both men and women around the world, especially in developing countries, where the prevalence of asymptomatic infection varies from 2 to 44%, depending on the population and studied region. It affects mostly basal epithelial cells of the skin or inner lining of tissues. 35 cervical brushings, collected from females with cytologically abnormal cervix. The target genes E6 and E7 of 235 bp were amplified by Conventional PCR. Three cases came positive having problems such as Discharge of pelvic fluid, Chronic pelvic Disease and Pain in lower abdominal and Discharge of pelvic fluid that means the early E6 and E7 genes of 235 bp of oncogenic Human Papilloma Viruses were Present. The maximum affective age for HPV infection is in between 25 to 35 mostly. So, before coming to this age every female needs to go for Pap test which looks for abnormal cells on the cervix and vaccination if female is not vaccinated. High risk HPV screening at an early stage will prevent further progression of the disease.

keywords: Oncogenes, Sexual Transmitted Diseases, Human papilloma Virus, Cervical brushing, DNA isolation, Vaccine.

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STD (Sexual Transmitted Diseases) are also known as venereal diseases. They are caused by microorganisms that live on the skin or in body fluids such as semen, vaginal fluid or blood. The microorganisms are passed from an infected person often through sexual contact with skin, blood or body fluids. These microorganisms can enter the body through the vagina, mouth, anus and open sores or cuts. The microorganisms can be Bacteria, Viruses and also Protozoa.

The most common types of Sexual Transmitted Diseases are: Gonorrhoea, Syphilis, Herpes, Chlamydia, Hepatitis B Virus, Human Immunodeficiency Virus, Human Papilloma Virus (HPV or genital warts) and Trichomoniasis. Each year, there are an estimated 357 million new infections with 1 of 4 STIs: chlamydia, gonorrhoea, syphilis and trichomoniasis¹⁰. Drug resistance, especially for gonorrhoea, is a major threat to reducing the impact of STIs worldwide. Cervical cancer is caused by human papilloma virus (HPV) infection. Human Papilloma Virus is a sexually transmitted infection (STI) that is very common among young men and women in many parts of the world. In addition, other indicators of sexual behavior and reproductive activities, heredity, immune and nutritional status, and smoking can contribute in some way to the development of cervical cancer^{8,7}. Human Papilloma Virus is a small, non-enveloped and double stranded DNA virus with a genome of approximately 8,000 nucleotides and with the diameter of 55nm. HPVs can also be classified as high-risk (HR-HPV) and low-risk (LR-HPV) oncogenic types. Infection with HR-HPV types, highlighting HPV 16 and 18, is associated with the occurrence of pre-malignant and malignant cervical lesions^{9,2,3}. Most HPV infection is transient and some studies show that the majority of sexually active individuals are exposed to and acquire infection from this virus at some phase in their lives^{1,13}. HPV can infect basal epithelial cells of the skin or inner-lining tissues and are categorized as cutaneous types or mucosal types. Human Papilloma Virus Genotypes were Characterized by Conventional Polymerase Chain Reaction (PCR) in females with Abnormal Cervix. Younger age^{6,11} and older age⁴, White race⁵, and higher levels of education⁵ were associated with increased knowledge of HPV. Similarly, African American race, non-White Hispanic race, and manual workers were associated with decreased knowledge of HPV¹⁴.

2. MATERIALS AND METHODS

Clinical specimens mainly cervical brushings were collected from females with abnormal cervix in virus transport medium. Further the DNA was extracted by using silica column method from all the specimens. Selection of the target Human Papilloma Virus DNA sequences has been based on the study of highly conserved regions in the HPV genome for Amplification of Early E6 and E7 oncogenic genes of **235 bp** which are responsible for Cervical cancer of the females. The master mix was prepared for Human Papilloma Virus 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. 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. In agarose gel

electrophoresis, the presence of HPV is indicated by a band of approximately 235 bp.

3. RESULTS

A total of **35** specimens (Cervical Brushing) were collected from females with cytologically abnormal cervix. The specimens were collected from Shri Mahant Indires 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 Conventional PCR. Amplicon size of 235 bp was obtained for Oncogenic Human Papilloma Virus.

From total samples, some samples are showing positive results who are having the problems such as Discharge of pelvic fluid, Chronic pelvic Disease and Pain in lower abdominal and Discharge of pelvic fluid and some samples are showing the negative result.

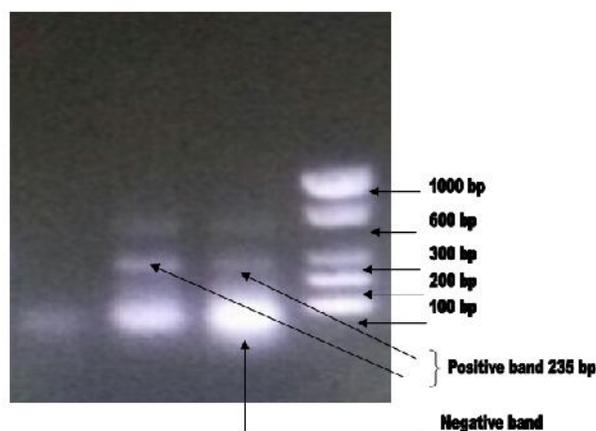


Fig 1: Positive band of HPV oncogenic gene by PCR Amplification.

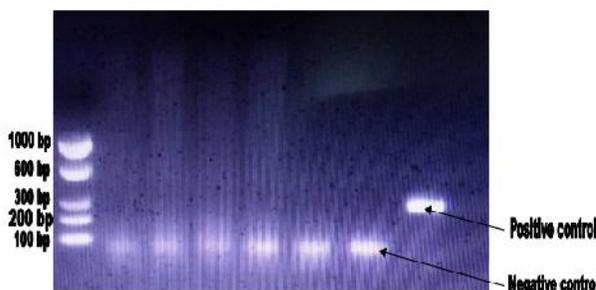


Fig 2: Negative band of HPV Oncogenic by PCR Amplification.

4. DISCUSSION AND CONCLUSION

During the past 30 years molecular techniques have been under development, however these have had a rapid and tremendous progress in recent year. Among molecular techniques, PCR and its different variations are highlighted as the most commonly used in laboratories and research institutes.

By using the Conventional PCR techniques, Early E6 and E7 genes of Human Papiloma Virus was Amplified. It have analysed that Cervical cancer can start to cause by HPV

from 20 years old age of female because 13 to 20 age of females are adolescences age when they get more sexually activated and most of cervical cancer cases were found to cause by the Sexual contact, oral sex, anal sex etc. Most HPV infection is transient and some studies show that the majority of sexually active individuals are exposed to and acquire infection from this virus at some phase in their lives. To come out from this infection, only vaccination is the way to protect health from infection. Vaccination is recommended for 11 to 12 year-old-girls. It is also recommended for 26 years of age who have not yet been vaccinated or completed the vaccine series. Otherwise the female who is for 30 age should go for pap test or screening test along with molecular level of test for cervical cancer. Pap test looks for abnormal cells on the cervix that could turn into cancer over time. If result will come as negative then no need to check up still 5 years. Gardasil Vaccine which is sterile preparation of intramuscular injection and contains purified inactive proteins from HPV types 6, 11, 16 and 18 and another one which is recently included by World Health Organization by India is Rotovac vaccine.

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