

Standardization of Human Papilloma Virus DNA Test Correlation with Pathology of Cervical Cancer

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Papilloma viruses are highly species specific, infecting Humans only. More than 70 types of HPV have been identified, some of which are thought to cause cancerous and pre-cancerous conditions of the cervix. In many countries, HPV is the most common sexually transmitted infection (STI). In women worldwide, cervical cancer remains the second most common cancer and that affects women mostly. In countries where screening is not implemented, cervical cancer is still a major health problem and a frequent cause of death. Cytology screening (e.g. Pap test) is the standard method used for the control of cervical cancer in India. Despite the availability of Pap testing even on an opportunistic basis in India, the incidence of invasive cervical cancer remains high, especially in rural India. Pap smear and DNA detection by PCR are test done to detect HPV infection. Out of 11 samples processed 4 were found to be HPV Positive and remaining 7 were negative which were in correlation with the Histopathological examination. PCR can help in early detection of HPV DNA; this can help in diagnosis and further treatment for HPV.

Key words: Human Papilloma viruses, Pap test, cervical cancer, PCR.

Cervical cancer begins in the cervix, the part of the uterus or womb that opens to the vagina. Cancer of the uterine cervix is the most common malignant tumor in women worldwide and represents a major public health problem in south-east Asia. Human Papilloma Virus has emerged as a major pathogen associated with

cervical cancer. Human papilloma virus is a virus that may cause warts on the hands and feet, as well as lesions of the mucous membranes of the oral, anal or genital cavities. Genital HPV types are sexually transmitted and cause external Genital Warts. More than 70 types of HPV have been identified, some of which are thought to cause cancerous and pre-cancerous conditions of the cervix.

There are two types of HPV- "low-risk" and "high-risk". Some low-risk HPV infections can cause genital warts. Some times, if the high-risk type of HPV does not go away on its own, it may cause abnormal, or pre-cancerous, cells to form. If these abnormal cells are not found and treated, they may become cancer. An HPV infection rarely leads to cervical cancer. Serological test such as Pap smear test is presently

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used to screen cervical scraps for the presence of HPV. At the same time Polymerase Chain Reaction, a Powerful and highly sensitive *in vitro* molecular biology technique, developed by Kary Mullis and his colleagues (1985), is used for the detection of target DNA for infectious diseases, cancer and genetic disorders in various clinical specimens.

PCR is a method for selectively and repeatedly replicating defined DNA sequences from a DNA mixture. Using PCR, the amount of new DNA generated increases geometrically with in few hours. Starting with one molecule of DNA, one cycle of PCR products two molecules, two cycles produce four molecules; and so on (Manos *et al.*, 1989 & Chua *et al.*, 1996). Detection of viral nucleic acid in paraffin embedded tissue by PCR is one of the reliable diagnostic methods for the laboratory detection of Human papilloma virus infection. Several factors determine the success of this highly sensitive method, including proper specimen preparation, use of optimal primers representing conserved regions of the viral genome, appropriate composition of the reaction mixture, and the thermal cycling profile (Forslund *et al.*, 1994).

Methodology

Isolation of DNA from paraffin embedded tissue

Transfer the paraffin embedded tissue (PET) to 1.5ml microcentrifuge tube and add 1ml of 1X PBS buffer. Repeat 2 to 3 times. Heat at 75° C for 5 minutes. Centrifuge at 12,000 rpm for 3 minutes and discard the supernatant.

Extraction of DNA

Add 50ml of DNA Extraction buffer, 2ml proteinase K to remaining pellet and mix well using pipette (Briefly mixing with vortex before adding DNA extraction buffer containing resin). Incubate at 56°C for 15 minutes. Vortex at high speed for 10 seconds. Place the tube in 100°C heat block or boiling water bath for 8 mins. Vortex at high speed for 10 seconds. Spin at 10,000-12,000 rpm for 2-3 minutes. Use 4.5ml of the supernatant for PCR reaction.

Primer Design

The present study employed a set of primers from conserved regions of HPV
 GP5 : 5' TTT GTT ACT GTG GTA GATACT AC 3'
 GP6 : 5' GAA AAA TAA ACT GTA AAT CAT ATT C 3'

Agarose Gel Electrophoresis

0.8 grams of Agarose is dissolved in 40 ml of 1XTBE buffer and is boiled in Micro oven. 2 ml of Ethidium bromide is added to the gel solution and mixed well. The gel solution is poured into a gel-casting tray and allowed to polymerize. The PCR products are mixed well with 5 ml of loading dye and then loaded in the wells along with 100 bp DNA marker and are run at 100-150 volts.

Analysis of the results

The gel is finally analyzed under UV-Transilluminator (Gel Documentation System) and Product size of 150 bp is observed.

RESULTS

The main aim of this project was to standardize a method to detect HPV (Human Papilloma virus) DNA in women. Among 11 patients analyzed 4 were found to be positive and remaining 7 were found to be negative by DNA PCR. (Fig.1). The PCR results shown in fig.1 are interpreted as follows: One of the thirteen samples is a known negative for a detectable HPV-specific PCR product (Lane 11) and another sample is a known positive sample for detectable HPV-specific PCR product (Lane 2). Four out of the thirteen samples (Lane 4, 7, 9) has been demonstrated to be positive for HPV DNA having an amplification product of size 150 bp indicative

Table 1. Detection of Human papilloma virus by PCR and compared with histopathological examination

S. No.	Sample No	HPV DNA Detection	Histo-pathology results
1	04-09699	-ve	-ve
2	04-09677A	+ve	+ve
3	04-09553A	-ve	-ve
4	04-09736	-ve	-ve
5	04-07518	+ve	+ve
6	04-08456	-ve	-ve
7	04-07681	+ve	+ve
8	04-09439	-ve	-ve
9	04-09226	-ve	-ve
10	04-07537	+ve	+ve
11	04-09538	-ve	-ve

of a infection with HPV and the remaining seven samples (Lane 3, 5, 6, 8, 10) has been demonstrated to be negative for HPV DNA by this PCR methodology. A 100 bp DNA Ladder is also loaded for reference (Lane 1).

The samples analyzed by PCR method were then correlated with the results obtained from histopathological examination done at the histopathology department. The four samples found to be positive and the remaining seven samples found to be negative (Table:1) showed similar results as that of HPV DNA detection by Polymerase chain reaction.

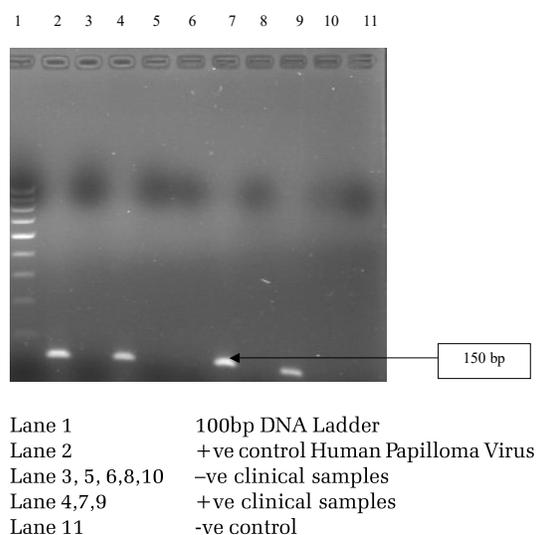


Fig. 1. Detection of Human papilloma virus by Polymerase chain Reaction

DISCUSSION

The development of HPV DNA detection holds tremendous promise for developing countries like India where cervical cancer is the most common malignancy among middle-aged women, particularly in the rural areas. The availability of HPV DNA detection will not only helps in curbing the cervical cancer incidence and high risk HPV DNA was done using PCR method Samples that were not confirmed by PCR had low viral load suggesting sampling error as a potential source of variability when sample viral loads are near the assay sensitivity threshold. Further, it is possible that the discrepancy between PCR and

histopathological examination. The good overall agreement is reflective of a large number of HPV negative samples; continued evaluation of HPV testing in this community with a larger sample size will be required to determine the relative performance of self- vs. clinician-collected samples for HPV testing. Self-sampling as a means to monitor HPV infection post-vaccination may prove to be a valuable tool in post-immunization surveillance in India. However, screening of larger samples size will give a better picture on the distribution of the HPV types.

Summary

Genital human papilloma virus infections are extremely common in young, sexually active women. While the majority of infections are inconsequential and short-lived, virus persistence is associated with anogenital disease, especially cervical carcinogenesis. While most HPV-associated lesions will remain benign or will regress spontaneously, approximately 15% of all HPV-positive cases will progress to high-grade cervical intraepithelial neoplasia and cancers, which almost invariably contain DNA of high-risk HPV types, e.g. HPV-16. Human Papilloma virus has emerged as a major pathogen associated with Cancer of the uterine cervix. Pap screening is the mode of cervical screening.

The biggest limitation of Pap test is the poor sensitivity (50-80%) and the need for frequent repetition. Thus standardization of PCR is very important in order to prevent false positive results during the process. Thus in the present study, standardization of DNA PCR of HPV correlates with cervical cancer is done. Out of 11 samples collected from Histopathology archives. DNA were isolated and amplified invitro by using PCR under appropriate conditions. Out of 11 samples processed, 4 were found to be HPV Positive and remaining 7 were negative which were in correlation with the Histopathological examination.

Establishment of HPV infection status and detection of HPV in clinical samples are gaining ground as important prognostic indicators in the clinical screening and management of those found to be at risk for the development of cervical cancer. In conclusion, PCR can help in early detection of HPV DNA; this can help in diagnosis and further treatment for HPV.

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