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Original Research Article

Molecular Revealing of Human Papilloma Viruses (HPV) in Abnormally Pap Smears by RFLP-PCR

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Abstract: The study was aimed to characterize genotypes of Human Papilloma Virus in abnormally Pap test. Out of 15 specimens processed, 02 came positive for oncogenic HPV. Both the positive cases were HPV type-16 which is one of the most important oncogenic type and with findings of LSIL and HSIL in those cases. Both the positive cases were seen in the age group 31-40 years. Such findings are of utmost clinical relevance for the disease management.
Keywords: Low grade squamous intraepithelial lesions, High grade squamous intraepithelial lesions, Oncogenic, Genotype, and Polymerase Chain Reaction.

INTRODUCTION:

The Human Papillomavirus (HPV) causes skin and *mucous membrane* infections, spreads through sexual contact and can transmit from one person to another by skin-to-skin contact. There are about 100 types of HPVs that can affect different parts of the body. Some types of HPV can cause warts such as genital or plantar warts and others can lead to cervical cancer or anal cancer [1]. The different HPV types are classified into low and high risk based on their association with clinical manifestations [2, 3]. In India, the number of people with uterine cervix cancer is rising, but overall the age-adjusted rates are decreasing [4-6]. This study characterizes the Oncogenic Human *Papilloma* virus genotyping in cytological abnormal females, followed by Restriction fragment length polymorphism (RFLP) for HPV type detection.

MATERIALS AND METHODS:

A total number of 15, cervical brushing from the patients with Low grade squamous intraepithelial lesions (LSIL), High grade squamous intraepithelial lesions (HSIL), vaginal bleeding and discharge etc. were collected from the Department of Gynecology and Obstetrics of Shri Mahant Indiresh Hospital, Patel Nagar, Dehradun, Uttarakhand (U.K). DNA from all the samples were isolated by silica column method and the isolated DNA were utilized for oncogenic early genes detection mainly *E6* and *E7*. The analysis of amplification products was performed by Agarose gel electrophoresis. After amplification, sample containing generic HPV DNA sequences will render a band of approximately 450 bp, while sample containing oncogenic HPV genotype will also render a band of 250 bp (Fig.1).

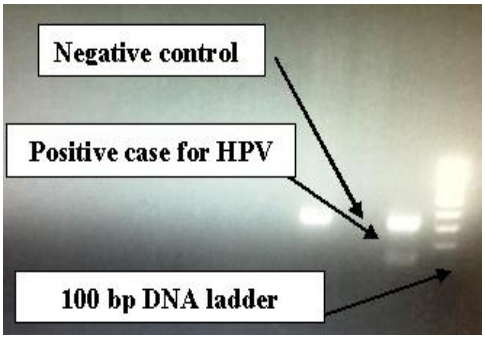

Fig 1: Gel picture for Oncogenic HPV

Table 1: Restriction pattern for the 250bp fragment for oncogenic genotype

	HPV16 238bp	HPV18 268bp	HPV31 232bp	HPV33 244bp	HPV35 232bp	HPV52 231bp	HPV58 244bp	HPV67 240bp
Digestion A	NC	NC	NC	NC	NC	NC	126, 118	NC
Digestion B	167,81	172,90	NC	137,138	NC	NC	NC	NC
Digestion C	NC	NC	NC	NC	NC	176,55	NC	NC
Digestion D	NC	NC	NC	NC	180,45	NC	NC	NC
Digestion E	NC	NC	117,118	NC	NC	NC	NC	NC

RESULTS:

Out of 15 specimens processed, 02 came positive for oncogenic HPV. Further the positive cases were digested with a set of different restriction endonucleases enzymes for HPV genotyping. both the positive cases were of Pap smears findings as LSIL and HSIL and when further processed for genotyping, yielded HPV type-16. As HPV is a sexually transmitted infectious agent, 02, positive cases were seen in the age group 31-40 years. It was seen that HPV type-16 was found in both the positive cases which is one of the most important oncogenic type (Table 2 &3).

Table 2: Pap smear status and other abnormalities (HPV Genotype Detected)

Sr. No.	PAP smear status and other abnormalities	HR-HPV PCR Result	HR –HPV Genotyping Detected
1	HSIL	Positive	HPV 16
2	LSIL	Positive	HPV 16

Table 3: HPV genotypes distribution according to age group

Age in years	Total cases	Hr-HPV positive	Hr-HPV negative	Genotype/s Detected
0-20	0	00	0	None
21-30	4	0	4	None
31-40	6	2	4	HPV-16
Above 41	5	0	5	None

DISCUSSION AND CONCLUSION:

Human Papilloma virus is primary cause of cervical cancer. Diseases caused by infection by HPV vary from condyloma to neoplastic transformation in cervix, vagina and vulva, as well as carcinoma [7-9]. Cervical cancer is the third or fourth most common female Malignancy worldwide, causing an approximate 529,828 new cases each year. Transmission of HPV by direct skin to skin contact, including sexual intercourse, anal sex, or and contact involve the genital area (hand to genital contact.) When virus persists (in 10 to 20% of cases) there is a chance of developing cervical cancer [10]. Study signifies about the routine screening of cervix, as most of the chances of getting HPV are in the age group of 31-40 years, which is the most productive and sexually active span of in females. High risk HPV type-16 was seen in both the positive cases reconfirming the prevalence of Oncogenic HPVs in the cases with abnormal pap smears.

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