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Human Papilloma Virus Detection Using Primers *MY09/MY11* in Cases with Cytological and Colposcopic Changes in Abnormal Cervix

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Abstract **Original Research Article**

Background: Human Papilloma Virus is one of the most common sexually transmitted infections. HPV is usually harmless and goes away by itself, but some types can lead to cancer or genital warts. All womens are at risk for cervical cancer it occurs most often in women over age 30. This study aimed to investigate HPV infection in women with cytological and/or colposcopic abnormalities in Uttarakhand population and also verify the performance of *MY09/MY11* primers for HPV detection. ***Materials and Methods*:** For this proposed study we collected the clinical specimens, and the study was conducted from the samples collected from Shri Mahant Indiresh hospital, Dehradun which includes OPD and IPD of gynaecology and obstetrics**.** ***Results*:** The average age of the studied population was 36 years (range: 18-58years). Cytological results, 03 (3.12%) cases ASCUS, 45 (46.87%), NILM, 28 (29.16%) cases inflammatory smear, 04 (4.16%), Bacterial vaginosis, 02 (2.08%), Atrophic vaginitis, 03 (3.12%), Unsatisfactory smear, 01 (1.04%) cases Post-menopausal smear & 06(6.25%) cases HSIL for squamous cell carcinoma showed positive colposcopy. Only 06 (6.5%) patient had HSIL and a positive sample to HPV, which demonstrated the effectiveness of PCR as standard molecular method. ***Discussions and Conclusion*:** This study suggests that more than one type of oligonucleotide primer should be used in clinical samples to increase the sensitivity in HPV detection. For the proper management and follow up protocols for the cases with positive HPV infections, such studies are clinically, relevant.

Key words: Cervical Cancer, cytology, colposcopy, High risk HPV, PCR.

INTRODUCTION

Human Papilloma Virus is one of the most common sexually transmitted infections. HPV is usually harmless and goes away by itself, but some types can lead to cancer or genital warts [1]. All womens are at risk for cervical cancer it occurs most often in women over age 30. Cancer of the cervix uteri is the 3rd most common cancer among women worldwide, with an estimated 569,847 and 311,365 deaths in 2018 (GLOBOCAN) [2]. About 96,922 new cervical cancer cases are diagnosed annually in India. Cervical cancer is the 2th most common female cancer in women aged 15 to 44 years in India [3]. Epidemiological and molecular studies have shown close link between human papilloma virus (HPV) and the onset of cervical cancer [4]. Molecular diagnosis of HPV infection is important for virus screening, and is mainly based on methods such as: hybrid capture (CH2), *in situ* hybridization, and PCR [5]. These techniques have varied widely in terms of sensitivity and specificity, and PCR is widely used in various areas of molecular diagnostics due to its great ability to detect small fragments of deoxyribonucleic acid (DNA). The system uses *MY09/MY11* primers, that amplify the L1 region of viral genome, are more frequently used for HPV detection in clinical and histological studies. These primers are effective for amplifying wide spectrum of HPV genotypes in cells obtained from cervical smears and paraffin-embedded tissues[6]. The pair of oligonucleotides *MY09/MY11* primers flanks a

sequence of approximately 450 bp. The pair *MY* is synthesized from several degenerate nucleotides in each primer, it is a mixture of 25 primers oligonucleotides able to amplifying > 25 genital HPV types.

Colposcopic examination for abnormal cell/Pap smear: The first step in finding cervical cancer is often an abnormal Pap test result. This will lead to further test which can diagnose cervical cancer [7]. The Pap test is a screening test, not a diagnostic test. It cannot tell for certain if you have cervical cancer [8]. An abnormal Pap test result may mean more testing; sometimes including tests to see if a cancer or a pre-cancer is actually present [9]. The tests that are used include colposcopy (with biopsy), after a biopsy the tissue sample is examined under a microscope to look for changes or abnormalities such as cancer. If there are no abnormal cells, the result is reported as normal [10].

This study aimed to investigate HPV infection in women with cytological and/or colposcopic abnormalities in Uttarakhand population and also verify the performance of *MY09/ MY11* primers for HPV detection.

MATERIALS AND METHODS

This is a cross-sectional study, performed during January to May 2019, from epidemiological data on cytological and/or colposcopic abnormalities cases diagnosed at Central Molecular Research Laboratory (CMRL) Dehradun. For this proposed study we collected the clinical specimens, and the study was conducted from the samples collected from Shri Mahant Indiresh Hospital, Dehradun which includes OPD and IPD of gynaecology and obstetrics [11]. The study included women had as cytological result: atypical squamous cells of undetermined significance (ASC-US), atypical glandular cells of undetermined significance (AGUS), low-grade intraepithelial lesion (LSIL), high-grade intraepithelial lesion (HSIL), and invasive carcinoma sample were collected in cervical sample with cytobroom viral transport media (VTM) and stored at -20ºC until DNA extraction.

DNA Extraction and Amplification

DNA extraction from cervical sampler (with cytobroom viral transporter media) was performed using QIAamp DNA Mini Kit (Qiagen Ltd, Crawley, UK), according to manufacturer's instructions. Aliquots of 200µl of samples were digested with 25µl of Proteinase K and 200µl of lysis buffer at 56ºC, for 15 minutes. DNA precipitation was performed by adding 500µl of ethanol (70%) (12) DNA was eluted in 80µl of elution buffer and amplification was done by using primers *MY09/MY11* with sequencing *MY09*(5′CGTCCMARRGGAWACTCATC3′), *MY11* (5'GCMCAGGGWCATAAYAATGG3') at 450 bp with cyclic condition at Denaturation 95ºC/20 second, annealing 56ºC/1minute, Extension 72ºC/1 minute were amplified. Internal control β-actin gene with sequencing β-actin gene(F)(5'TCACCCACACACTGTTGCCATCTAGA3'),βactin(R)(5'CAGCGGAACCGCTCATTGCCAATGG 3'), at 306 bp amplified for HPV screening (Table no. 1).

Table-1: Primer selection for *MY09/MY11*,β actin gene

Primer	Sequence	Amplicon size	Protocol (PCR)	Conditions
MY09 MY11 β actin gene (F) β actin gene (R)	5′CGTCCMARRGGAWACTCATC3′ 5'GCMCAGGGWCATAAYAATGG3' 5'TCACCCACACACTGTTGCCATCTAGA3' 5'CAGCGGAACCGCTCATTGCCAATGG3'	450bp 306bp	Buffer 5µl, dNTPs 1.5µl, $MgCl_2$ 2µl MY09 0.5µl MY11 0.5µl, βactin1 0.5 µl, βactin2 0.5, Red Taq 0.5µl NFW 9µl,	40cycle: Initial denaturation 95ºC/3min Denaturation 95ºC/20sec Annealing 56ºC/1min Extension 72ºC/1min Final extension 72ºC/10min

RESULTS

A sample survey of cytological and/or colposcopic abnormalities cases diagnosis was performed. The average age of the studied population was 36 years (range: 18-58years). Cytological results, 03 (3.12%) cases, ASCUS 45 (46.87%), NILM, 28 (29.16%), inflammatory smear, 04 (4.16%), Bacterial vaginosis, 02 (2.08%), Atrophic vaginitis, 03 (3.12%), Unsatisfactory smear, 01 (1.04%) cases Post-menopausal smear & 06(6.25%) cases HSIL for squamous cell carcinoma showed positive colposcopy. Only 06 (6.5%) patient had HSIL and a positive sample to HPV, which demonstrated the effectiveness of PCR as standard molecular method. (Table no.2) According to age wise distribution, 01 (1.04 %) case was positive, 10 (10.41) % cases were negative in 20-30 year of age group. 03 (3.12%) cases were positive, 37 (38.54%) cases were negative in 31-40 year of age group. 02 (2.08 %) cases were positive, 33 (34.37%) cases were

negative in 41-50 year age of group. 0 % cases were positive & 07 (7.29 %) cases were negative in 51-60-year of age group. 0% cases were positive, 03 (3.12%) cases were negative for above 60 year. Out of 96 cases 06 (6.25%) were positive and 92(93.75%) cases were negative for high risk HPV. And 04 (4.16%) cases were positive, 92 (95.83%) were negative by using primers *MY09/MY11* respectively (Table No.4).

Table-2: Colposcopic examination for abnormal cell

S.No	Pap smear status	Total sample	High risk HPV positive for E6 and E7 genes	PCR for HPV positive by *MY09/MY11* Primers
1	Negative for intraepithelial lesion or malignancy (NILM)	45 (46.87%)	00	02 (2.08%)
2	High-grade squamous intraepithelial lesion (HSIL)	6 (6.25%)	06 (6.25%)	01 (1.04%)
3	Atypical squamous cells of undetermined significance (ASCUS)	3 (3.12%)	00	00
4	inflammatory smear	28 (29.16%)	00	01 (1.04%)
5	Bacterial vaginosis	4 (4.16%)	00	00
6	Atrophic vaginitis	2 (2.08%)	00	00
7	Unsatisfactory smear	3 (3.12%)	00	00
8	Post-menopausal smear	1 (1.04%)	00	00
9	Limited NILM with bacterial vaginosis	3 (3.12%)	00	00
10	Suspicious of squamous cell carcinoma	1 (1.04%)	00	00
	Total no. of sample	**96**	**06 (6.25%)**	**04 (4.16%)**

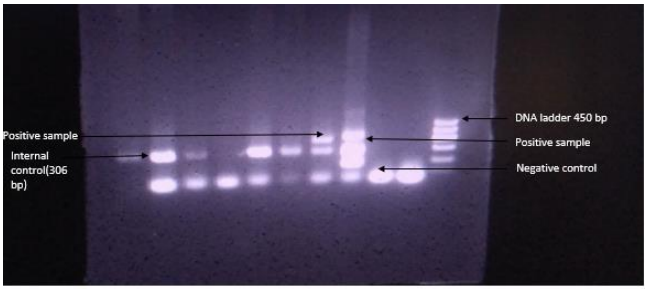

Fig-1: Agarose electrophoretic image gel picture showing HPV type at 450 bp using *MY09/MY11* primers and β actin gene used as IC at 360 bp

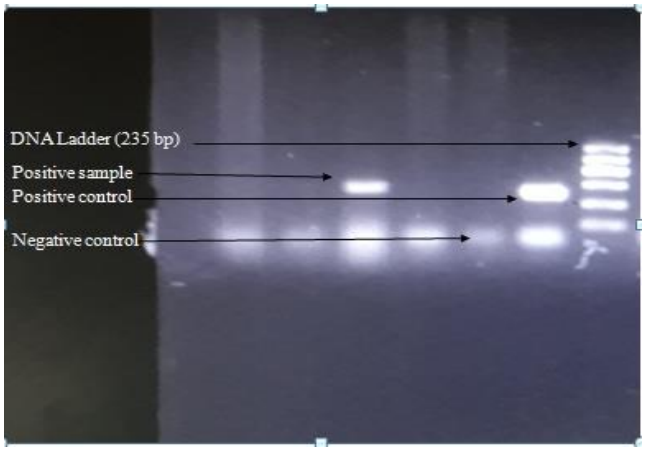

Fig-2: Agarose electrophoretic image gel picture is showing oncogenes(E6 and E7) high risk HPV at 235bp

Table-3: Total number of Human papilloma virus (HPV) infection cases

Total number of cases	Positive	Negative
96	6 (6.25%)	90 (93.75%)

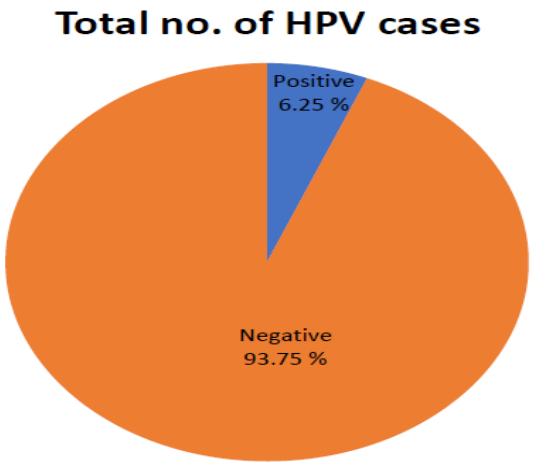

Fig-3: Pie chart depicting HPV positive and negative cases

Table-4: Age wise distribution for HPV infection cases

Age in year	Number of cases	Positive cases	Negative cases
20-30	11 (11.45%)	1 (1.04%)	10(10.41%)
31-40	40 (41.66%)	3(3.12%)	37 (38.54%)
41-50	35 (36.45%)	2 (2.08%)	33 (34.37%)
51-60	7 (7.29%)	0	7 (7.29%)
Above 60	3(3.12%)	0	3 (3.12%)
Total no. of cases	**96**	**6(6.25%)**	**90 (93.75%)**

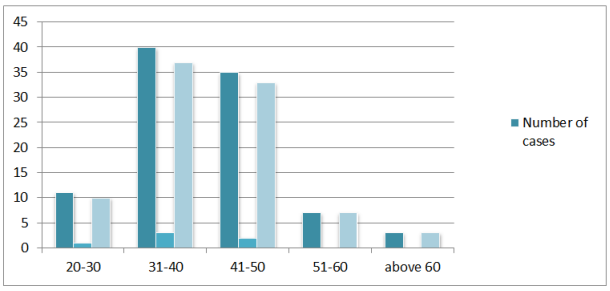

Fig-3: Age wise distribution of human papilloma virus in different Age groups

DISCUSSION

HPV is recognized as a public health problem for its role as a crucial factor in pathogenesis of various cancer. Cervical cancer is a preventable disease [13]. It develops following progression of uncleared HPV infection to high grade & eventually to invasive disease women with normal cervical cytology, who are infected with high risk of developing CIN 3, compared with uninfected women [14]. Persistence of onogenic HPV appears essential for the development of cervical cancer. *MY09/MY11* oligonucleotide primer was used in this study to HPV detection. For the PCR reaction a Positive control is used (DNA extracted from positive HPV sample), and negative control is used (H_2O) the result was considered positive for DNA-HPV when MY09/MY11oligonucleotide primer testing Detected the viral DNA. Infection with specific subtypes of HPV has been strongly implicated in cervical carcinogenesis. The identification and functional confirmation of host proteins associated with HPV E6 and E7 oncoproteins may provide useful information in understanding cervical carcinogenesis and the development of cervical cancer-specific markers. PCR is being progressively more used in clinical laboratories to HPV diagnose. Oligonucleotides primers commonly used *MY09/MY11* detect a wide range of HPVs, using annealing temperature (56ºC) & can amplify multiple HPV infections. However, E6 and E7 primer has (62 ºC) & (58 ºC) anealing temperature. Studies in literature indicate variables rate of HPV detection. These differences in detecting DNA of HPV suggest a potential difference in detecting DNA of HPV suggest a potential difference in the ability to amplify fragments of different sizes and specific types of HPV. Our aim was to evaluate the Polymerase chain reaction (PCR) technique, using primer *MY09/MY11* for screening of HPV. A total of 96 samples was evaluated for the diagnosis of HPV, Firstly DNA was isolated by spin column method & then subjected to amplification by PCR after completion of amplification, post amplification was done gene was detected under UV transilluminator by agarose gel electrophoresis [15]. in which Gel picture is showing HPV type at 450 bp using

MY09/MY11 primers and β actin used as IC at 360 bp (Figure no.1) & Gel picture is showing oncogenes (E6 and E7) high risk HPV at 235bp (Figure no. 2). In our study, HPV E6 and E7 gene were detected for cervical cancer 6 samples (6.25%) came positive and 90 samples (93.75%) came negative for high risk HPV whereas for HPV Screening using *MY09/MY11* primers 4 samples (4.16%) came positive & 94 samples (95.83%) came negative for HPV screening. According to age wise distribution highest no of cases found in age group of (31-40) year, total 40 cases (41.66%) in which 3 (3.12%) cases were positive and 37 (38.54%) cases were negative and lowest number of cases found in age group of above 60, total 3 (3.12%) cases in which zero positive cases and 3 (3.12%) negative cases were found. *MY09/MY11* primers used for just screening of HPV has less number of positive cases 4 (4.16%) in comparing to E6 and E7 used for high risk HPV with 6 (6.25%) positive cases out of 96 samples.

CONCLUSION

Considering the great intra- and inter-observer variability associated to cytological interpretation of preneoplastic and neoplastic cervical lesions, the use of molecular detection methods, such as PCR technique, becomes an important device in identifying patients with HPVs responsible for developing cervical neoplasia, thus supporting medical management. This study suggests that more than one type of oligonucleotide primer should be used in clinical samples to increase the sensitivity in HPV detection. For the proper management and follow up protocols for the cases with positive HPV infections, such studies are clinically, relevant.

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