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Genotypic Detection of HPV 16 and HPV 18 in Cases of Cervical Cancer from Southern Odisha, India

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KEYWORDS

Human Papilloma Virus; Cervical Cancer; Polymerase Chain Reaction; HPV 16; HPV 18

ABSTRACT:

Introduction: Human Papilloma virus (HPV) is one of the major risk factors for the occurrence of cervical cancer (CaCx) globally including India. The prevalence of HPV among the patients with CaCx is found to be varied.

Objectives: This study was carried out with an objective to find out the prevalence of HPV 16 and HPV 18 in CaCx samples in southern Odisha, India.

Methods: Cervical tissues samples were collected and subjected to histopathological analysis. Histopathologically positive samples were further evaluated for HPV infection by PCR method with little modifications with an initial heat treatment during DNA isolation. Two subtypes of HPV (HPV 16 and HPV 18) were investigated in this study. Description analysis was done for the presentation of generated data.

Results: A total of 122 positive cases, confirmed by histopathology for CaCx were considered for DNA isolation followed by HPV 16 and HPV 18 subtype identification. Tissue samples with a lysis step of 2-4 hours incubation at 100˚C were significantly resulted with good yield of DNA. About 98 cases (80.32%) were found to be positive for HPV infection including HPV16 in 80 (81.63%) cases, HPV18 in 16 cases (16.33%) and both HPV 16 and HPV 18 in two cases (2.04%).

Conclusions: The prevalence of HPV infection was found to be high in women diagnosed with CaCx in southern Odisha, which is a warning alert for the early HPV vaccination as well as early diagnosis of HPV infection.

1. Introduction

Cervical cancer (CaCx) is the fourth most common cause of mortality in women worldwide, with 662,301 new cases and 348,874 fatalities as reported by Kulkarni et al. (2023)[1]. About 127,526 new cases arise and cause 79,906 deaths each year, which makes it the third-most common cancer in India [2]. In India, CaCx occur around 1 in 53 women in contrast to 1 in 100 women in more developed regions of the world [3]. The major risk factors associated with CaCx are age, multiple pregnancies, number of abortions, and use of oral contraceptives as well as Human papillomavirus (HPV) infection. The relationship of HPV and CaCx is as high as association of smoking with lung cancer [4-9].

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HPV is very common worldwide and primarily transmitted through sexual contact in both males and females [10]. Approximately, 90% of HPV infections are asymptomatic and cleared by immune system. Epidemiological data and molecular observations have revealed that a persistent infection with high-risk HPV is the most crucial risk factor for cervical, genital and other organ site cancer. HPV infection is the key etiologic factor for CaCx, with more than 200 sub-types already identified and classified according to their oncogenic potential [11]. Around 40 HPV sub-types are found in the genital tract that is passed through sexual contact. About 10 HPV sub-types including HPV 16, HPV 18, HPV 31, HPV 33, HPV 35, HPV 45, HPV 51, HPV 52, HPV 58 and HPV 59 have been adequately evaluated as high-risk types or oncogenic types [12]. However, HPV 16 and HPV 18 sub-types are most frequent, accounting for up to 83.2% of all cases of invasive CaCx worldwide [13-14].

HPV being an important risk factor, it needs to be studied in every part of the country as the incidence of HPV and its subtypes are different in various geographical regions of the country. The World Health Organization (WHO) has advised to inspect for HPV as it can be a greatest CaCx prevention measure, particularly in low and middle-income nations [15]. There are many methods for the screening or diagnosis of HPV, but the molecular diagnosis is the gold standard methods. The molecular confirmation is important for the characterization of sub-types. Further, there is an urgent need for molecular-epidemiologic studies in regions that have verified the strong association between persistent infection by specific oncogenic-type HPVs and its relation with CaCx [1].

2. Objectives

This study has been planned with the aim to evaluate the prevalence of high-risk HPV types i.e. HPV 16 and HPV 18, in archival cervical biopsy samples diagnosed with CaCx in a tertiary health care hospital in southern Odisha, India

3. Methods

This was a cross-sectional study and carried out in women attending Departments of Obstetrics and Gynaecology (O&G) of M.K.C.G. Medical College and Hospital, Berhampur, Odisha, India. After physical observations, cervical tissues suspicious for CaCx were collected and were sent to Department of Pathology for histopathological analysis. Histopathologically positive samples were further evaluated for HPV infection by PCR method in the Multi-Disciplinary Research Unit (MRU), of M.K.C.G. Medical College and Hospital, Berhampur, Odisha, India

The collected tissue samples were stored in HiViral transport medium (Hi-Media Laboratory Pvt. Ltd., Maharashtra, India) and stored in -20°C before DNA isolation. Around 25 mg of tissues cut into small pieces and taken in a 2 mL centrifuge tube for DNA isolation using HiPurA® Mammalian Genomic DNA Purification Kit (Hi-Media Laboratory Pvt. Ltd., Maharashtra, India) as per manufacturer's instructions with minimal modifications for better yield. In details, after adding lysis buffer (AL), the tissue was incubated at 100°C (recommendation of 55˚C) for 2-4 hours by mixing of emulsion in 15 minutes apart. After incubation, another lysis (C1) buffer was added along with proteinase K and the mixture was incubated at 70°C for 3-4 hours (recommendation of 10 minutes) for further processing. Isolated DNA samples were quantified using Nano-Drop (DS-11+ Spectrophotometer; DeNovix) and diluted according to the requirement for PCR. For initial standardization procedure, the DNA quality and quantity have been checked and compared in 10 initial samples.

PCR was carried out as per the PCR conditions described by Ghose et al. [16], with little modification, in details, initial denaturation at 95°C for 10 min, 30 cycle of denaturation at 95°C for 30 sec, annealing at 58°C for 30 sec and elongation at 72°C for 30 sec followed by a final extension at 72°C for 30 sec. Both sets of primers designed for HPV16 and HPV18 were checked in NCBI database. The primer set for the detection of HPV 16 were selected from the L1 region of the HPV genome (locus LC456607; region 5937-6182);forward primer (5'-GCCTGTGTAGGTGTTGAGG-3') and reverse primer (5'-TGGATTTACTGCAACATTGG-3') amplified a 246 base pairs product. Similarly, the primer sets for the detection of HPV 18 were selected from E1 region of the HPV genome (locus MF288726; region 2522 - 2661) Forward primer (5'-GTGGACCAGCAAATACAGGA-3') and reverse primer (5'-TCCAACACGTGGTCGTTGCA-3')

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amplified a 162 base pairs product. The amplified products were separated in 2% agarose gel and documented using Gel-Doc XR+ documentation system (Bio-Rad Pvt. Ltd., CA, USA)

The generated data (descriptive) was presented in number followed by percentage. This study was approved by the institutional Ethical Committee of M.KC.G. Medical College, Berhampur, Odisha (No 705/Chairman-IEC, M.KC.G. Medical College, Berhampur-04; Date 14-08-2019).

4. Results

This was a cross-sectional study includes 128 number of CaCx tissue samples for the investigation of HPV infection. The DNA was isolated from 122 numbers of samples successfully with little modification of the earlier literatures. The modification included a heat treatment (100˚C) during the lyses steps for 2-4 hours instead of recommended 55˚C resulted with good quality and quantity of DNA. The mean of DNA quantity of the actual and modified procedures of 10 initial samples was found to be 11.65±3.35 ng/uL and 22.47±1.43 ng/uL respectively.

The detection of HPV 16 and HPV 18 infection was carried out by separate PCR. Among the 122 CaCx samples, 98 (80.32%) were found to be positive for HPV infection including HPV 16 only in 80 (81.63%) cases, HPV 18 only in 16 (16.33%) cases and rest two (2.04%) cases had both HPV 16 and HPV 18. The prevalence of HPV 16 (82.65%) was found to be higher as compared to HPV 18 (17.35%). The detail detection of both HPV 16 and HPV 18 in all the samples has been shown in Figure 1.

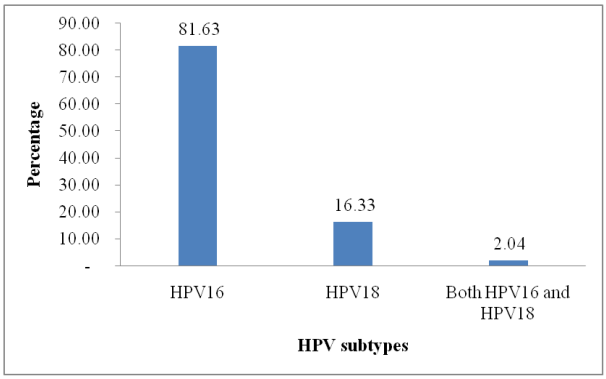

Figure 1: Prevalence of HPV and its subtypes in the study samples (n=122)

5. Discussion

In this cross-sectional study, 122 number of CaCx tissue samples were investigated and the prevalence of HPV infection was found in 80.32% (98/122) of samples. In the current study, both HPV 16 and HPV 18 have been detected with the prevalence of 82.65% and 17.35% respectively. The prevalence of HPV was found to be varied in many previous studies undertaken globally including India. A study conducted from the archives of eight institutes in four different regions of Thailand (northern, southern, north-eastern, and central) by considering 410 formalin fixed paraffin-embedded cervical tissue samples showed the prevalence of HPV infection of 88.8% whereas HPV 16 and HPV 18 had prevalence of 83.2% and 59.3 % respectively [17]. Another study was carried out in Ghana, where about 256 cases were considered and the prevalence for HPV was found to be 89.8%. Here, the authors reported 4 subtypes of HPV infection including HPV 16 in 10% of cases and HPV 18 in 47.4% of cases [18]. In a retrospective cross-sectional study undertook in 60 paraffin-embedded samples in Brazil, overall prevalence of HPV infection was 57.9%. In this study, HPV 16 and HPV 18 were found to be positive in 17.5% and 7% respectively with co-infection of 75.4% of cases [19].

In continuation with the global report on the prevalence of HPV in CaCx, there were inconsistence findings on prevalence of HPV as well as subtypes in India. A study was conducted in 684 cervical lesions considering both normal and abnormal cytology findings, the prevalence of HPV infection was reported in 36.84%. Among these positive samples, HPV 16 and HPV 18 together were predominating (79.37%) compared to others subtypes [20]. Similar observation has also been reported by another study carried out in Indore, India, considering 21-60 years of women [1]. In a retrospective study on cervical tissue biopsies collected from 110 patients with cervical abnormalities in Haryana, HPV was reported in 97.27% of cases with 90 (84.11%) samples testing positive for HPV 16 and 78 samples (72.89%) were positive for HPV 18 [21]. In another study from Gujarat, India, out of 400 samples analyzed, 74% of cases were positive for overall HPV infection, 67% had HPV 16, 14.8% had HPV 18 and 7.7% had both HPV 16 and HPV 18 [22]. In a study from the state of Odisha, India, about 607 numbers of

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participants have been analyzed for the HPV detection and genotyping. The overall prevalence of HPV infection was 60.33% (359/595). Further the authors reported the most prevalent strain was HPV 16 (87.28%) followed by HPV 18 (24.56%) and co-infection for HPV 16 and HPV 18 was 4.32% [9].

The findings of the present study along with other previous studies from different geographical regions revealed a changing spectrum in the prevalence of HPV infection as well as the prevalence of both HPV 16 and HPV 18 subtypes. Interestingly our findings are found to be similar with an earlier study carried out in the state of Odisha, India [9]. This might be due to the inclusion of cases from same geographical area. The differences in the prevalence of HPV in many studies may be explained for the inclusion of cases from various geographical regions, storage of samples, as well as method of diagnosis. In this present study, a heat treatment of tissue samples during the initial lysis step resulted with good yield of DNA that also enhanced the accuracy of PCR for the detection of HPV. Similar results were also obtained by adding a preheat treatment in different tissue samples during DNA isolation in other studies [23-24].

In conclusion, around 80% of cases were positive for HPV infections indicating a high prevalence of HPV infection in the study area. This alarming situation calls for an early vaccination as well as early diagnosis of HPV infection. The observations from this study cannot ignore the presence of other sub types of HPV as we have not investigated them in our study. Secondly, an initial heat treatment in lysis step may be considered for better yield of DNA during DNA isolation especially from the tissue samples. This will also enhance the better diagnostic approach for the detection of any genome associated markers in the tissues samples.

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Authors contribution: MVN, PKD, PP and NK designed the research, MVN, PKD and PP carried out all laboratory investigations; PP and NK analyzed the data; MVN, AN and PP wrote the manuscript; All the authors approved the final version of the manuscript.

Conflict of interest: None

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