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Research Article

REPORT OF HUMAN PAPILLOMAVIRUS 16 AND 18, CAUSING CERVICAL CANCER FROM RAYALASEEMA REGION, ANDHRA PRADESH, INDIA

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ABSTRACT

In the present study, we attempted the partial characterization of HPV 16 and HPV 18 which is responsible for the cervical cancer in Rayalaseema region of Andhra Pradesh, India.

INTRODUCTION

Throughout the world and also in developing countries like in India many women from age 16 to 55 are under the risk of Cervical Cancer. The development of cervical cancer after an HPV infection involves a number of other circumstances, including gene mutations, and occurs many years after the initial HPV infection. Human papillomavirus is a necessary factor, but it is not sufficient for development of dysplasiaorcarcinoma. Disease generally develops when there is persistent HPV infection of the cervical epithelium (1) (2). No Early Warning Signs like other cancers, cervical cancer rarely shows signs in its early stages. Symptoms typically only become apparent when the cancer cells grow through the top layer of cervical tissue into the tissue below it and this is known as invasive cervical cancer. Vaginal Discharge along with bleeding, other types of vaginal discharge are common early symptoms of cervical cancer. It is often continuous because of the nature of the infection. The discharge may have the following characteristics: pale, watery, brown, foul-smelling, and tinged with blood. Advanced Symptoms are like more intense symptoms will arise in later stages. Symptoms of advanced cervical cancer can include: back or pelvic pain, difficulty urinating or defecating due to obstruction, swelling of one or both legs, fatigue, and weight loss (3). The genome is small, icosahedral, 7900bp, NE (Non-enveloped) and 5nm in diameter. In the present study, we attempted the partial characterization of HPV 16 (HPV 16 –TPT-1a) and HPV 18(HPV 18 –TPT 1a) which is responsible for the cervical cancer in Rayalaseema region of Andhra Pradesh, India and described its distinctiveness from, and phylogenetic relationship with other isolates and also the other serotypes which causes cervical cancer from different geographical locations with in the country and also from Asian American and European Countries, which proved to be ubiquitous in nature.

MATERIALS AND METHODS

Sample collection*:* The samples were collected from the Govt. Hospital and with the clearance of ethical committee from Sri Venkateswara Medical College. The samples were collected from the female patients with the help of the Gynaecologist. The sample was scraped from the cervix region by rotating the spatula to 360˚. The scraping is then evenly spread on to a glass slide, which was immediately fixed using 95% ethyl alcohol and ether to avoid air drying artifacts(4), before collecting the samples, a questionnaire was prepared and gave awareness about the cervical cancer and the association of Human Papillomavirus with cervical cancer. With the willingness of the female patients the samples were collected, the samples were sent Pap smear and for Molecular diagnostics (Polymerase Chain Reaction). The cervical scrapings are taken as a specimen for the diagnostic purpose. The samples were collected in the Phosphate Buffered Saline (PBS) which acts as both storage and transport media.

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Isolation of total DNA

The method which is used for the isolation of the total Nucleic Acid is the high salt method concentration. In this method 600 µl of TNES buffer (Combination of Tris, Nacl, EDTA) is taken in to an sterile eppendorf tube to this 500 µl of the suspected sample is taken and to this 35 µl of Proteinase-k (20 mg/ml) was added and incubated at 50˚C for overnight. The incubated samples were taken and to this 5N NaCl of 166.7 µl was added and it is kept at room temperature for 5 min and centrifuged the sample at 12000×g for 20 min at 20°C. Then the supernatant was taken in to another sterile Eppendorf and to this equal amount of cold absolute alcohol was added and incubated at room temperature for 5 min. The incubated supernatant is then kept for centrifugation at 12000×g for 20min at 4°c. The precipitate is formed in the bottom of the eppendorf, the supernatant is discarded carefully which should not disturb the precipitate formed at the bottom of the eppendorf to this 300µl of 70% alcohol is added then this is kept for centrifugation at 4°c for 5 min at 7500×g.The supernatant is discarded then the precipitate is dried for 20min up to the alcohol smell get vanished. Then the isolated DNA is dissolved by using 100µl of nuclease free water or Milli-Q water.(4)

Amplification of the Viral DNA by PCR*:* The isolated total DNA was used for amplification of the Viral HPV DNA. 10X taq buffer of 2.5mM, 25mM $MgCl_2$dntps, Forward primer, reverse primer, 2µl of the isolated DNA and 0.3µl of Taq.Polymerase (Fermentas), were optimised for the amplification of the viral DNA. Here twosets of Primers were designed, to the E6 region of HPV 16 and to E6 region along with LCR for HPV 18. PCR amplification conditions for two sets of primers included denaturation cycle of 5 min at 95˚c followed by 35 cycles of denaturation for 30 sec at 95˚c, here the annealing for 30 sec at 54˚c, 53˚c for HPV 16 and HPV 18 respectively, for 30sec at 72˚c with a final extension of 10 min at 72˚c using gradient thermal cycler (Corbett Research, Model CGI -96, Australia.)

Agarose Gel Electrophoresis*:* The resulted PCR product was analysed through 1% Agarose gel electrophoresis. The gel was visualised in gel document system UV Transilluminator (Alpha Innotech Co-operation).

Nucleotide sequencing*:* The PCR- amplified fragment were purified by using Invitrogen gel extraction kit according to the manufacturer's instructions (Invitrogen, Germany) And the gel eluted PCR product is further sent for sequencing to Eurofins, Bangalore, India. We sent our designed forward and reverse primers for sequencing.

RESULTS

Amplification of HPV 16 E6 gene at 348bp 1(a)

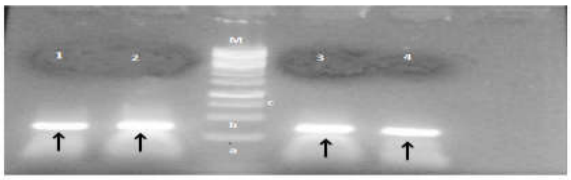

Fig 1(a) Resolution of 348bp in 1% agarose gel (Arrow mark indicates the amplicon size.) from lane 1,2,3 and 4 represents the positive samples for HPV 16 and M lane indicates the marker that is used, i.,e 1kb marker starting from 250bp(a), 500bp(b),750bp(c).

Amplification of E6 gene in HPV 18 at 510bp

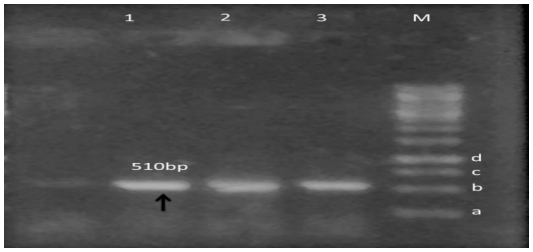

Fig 1(a) Resolution of 510 bp in 1% agarose gel (Arrow mark indicates the amplicon size.) from lane 1,2and 3 represents the positive samples for HPV 18 and M lane indicates the marker that is used, i.,e 1kb marker starting from 250bp(a), 500bp(b),750bp(c) and 1000bp(d)

DISCUSSION

The set of primers could amplify the desired 348bp of HPV 16, and for HPV 18 it is of 510bp .This proved the presence of virus in cervical scrapings suspected of Human Papillomavirus infection.). HPV-16 and HPV -18 are the serotypes which were found to be in high prevalence and are categorized as high-risk type of serotypes. The factors which are found to be the key role in the transmission of Human Papillomavirus were the parity, unhygienic conditions, early marriages in case of the developing countries, smoking, intake of OC pills. The early detection of HPV is to be improve in molecular approaches, serological approaches and also rapid test for HPV is very much necessary for the self-detection or easy identification of HPV. The most important is awareness about Cervical cancer, Human Papillomavirus and about pap smear is must for every women of above 16 years.

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References

1. Jhingran A, Russell AH, Seiden MV *et al*.2008 Cancers of the cervix, vulva, and vagina. In: Abeloff MD, Armitage JO, Niederhuber JE, *et al*. eds. *Abeloff's Clinical Oncology*. 4thed. Philadelphia, PA: Elsevier Churchill-Livingstone;: chap 91
2. Steenbergen RD, de Wilde J, Wilting SM, *et al*.2005 HPV-mediated transformation of the anogenital tract. *J Clin Virol*.;32(suppl 1):S25–S33
3. Suresh B. Cervical Smear collection procedures.1996 In Gynaecological cytology cervix. Is ted. New Delhi: Interprint:. P 141-52.
4. Walboomers JM, Jacobs MV, Manos MM, *et al*.1999; Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*. 189:12–19.

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