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Human Papilloma Virus (HPV) genotyping in the aetiology of Cervical Cancer: A pilot study from Visakhapatnam, India.

Dr. T. Santhi. MD[1]. Dr. B. Manjula MD[2,] Dr. J. Chandrika[3]
[1]Assistant Professor, Assistant Professor [2], PG student[3]
Dept. of DVL Andhra Medical College, Visakhapatnam – 530002.

Abstract: *The role of human papilloma virus (HPV) genotype in the pathogenesis and biological behaviour of cervical carcinoma of intense investigation. This study used PCR analysis to identify HPV in paraffin-embedded tissue and cervical scrapings from 50 patients with cervical cancer and 25-84 yrs/gender-matched controls population. Results were correlated with clinical outcome. HPV was identified in 48% of patients with cervical cancer and 4% of controls. The present study showed the presence of HPV type 16 in 40 patients (80%) where as HPV type 18 in 08 patients (16%) with cervical carcinoma. There was statistically significant association of HPV 16 with cervical carcinoma or HPV infection. Clinically, HPV-associated carcinoma was present in younger patients in comparison with age (mean age, 48.6* versus *56 years;* P = *0.001). The odds for patients with HPV infection to develop carcinoma were 20 times greater than for patients without HPV infection (95% confidence interval 4.1, 74.2). HPV is an independent risk factor for cervical cancer. Use of multiple specimen types or the development of highly sensitive and robust in situ hybridization HPV-testing methods to evaluate the certainty of attribution of lesions to HPV types might provide insights in future efforts, including HPV vaccine trials.*

Keywords: *Cervical Carcinoma, HPV, PET, PCR, vaccine*

I. Introduction

Cervical cancer is one of the commonest cancers of the female anogenital tract and a leading cause of morbidity and mortality. The association of HPV and cervical cancer was first suggested by zur Hausen in 1976.

It is now believed that 94-100% of cervical cancers - as well as tumours of the penis, anus, vagina, and vulva - are associated with sexually transmitted genital infection by the human papilloma virus (HPV)(2,3).There are at least 118 fully described forms of the Papilloma virus which structurally consists of double-stranded circular DNA surrounded by a viral capsid protein(4). Here we review how the genes of specific HPV genotypes interact with host cell DNA and protein to produce cervical epithelial dysplasia.

1.1. The HPV Genome - key players

The circular HPV DNA is 6800 to 8000 base pairs in length and codes for eight genes - E6, E7, E1, E2, E4, E5, L1 and L2. The first six are "early" viral genes which code for proteins produced during the early phase of infection in the basal cell layer. They result in enhanced proliferation of the infected cells and their lateral expansion (5). The E5 Protein has been shown to complex with epidermal- growth-factor receptor, platelet-derived-growth factor receptor and the colony-stimulating factor-1 receptor, which promotes growth (6). E5 also appears to inhibit programmed cell death (7). Nevertheless the fact that the viral E5 gene is often deleted during the process of viral DNA integration with the host cell genome suggests a dispensable role in oncogenesis. E6 and E7 genes and their proteins appear to have a central role in HPV-induced cervical cancer. They are expressed in cervical cancers and are individually able to immortalise various human cell lines in vitro but when expressed together their efficiency is enhanced (8). The E6 Protein has significant effects by virtue of its interaction with, and degradation of, p53 (9). p53 is also known as the "guardian of the genome" and is crucial in protecting normal cells when exposed to stress (e.g. radiation, UV light or chemicals). In such cells it causes cell cycle arrest preventing a cell with damaged DNA from multiplying, and allowing the cellular repair systems to fix any damaged DNA. If repair is not feasible then p53 induces apoptosis (programmed cell death). Since all cancers arise on a background of DNA mutations, p53 has a key role in preventing carcinogenesis and unsurprisingly 50-60% of all cancers have p53 mutations. Other effects of the E6 protein include degradation of the pro-apoptotic BAK protein which is involved in the intrinsic (mitochondrial) death pathway. BAK has a physiological role in the cellular response to stress, in that it can promote opening of the mitochondrial permeability pores releasing intra-mitochondrial cytochrome-c which induces apoptosis. E6 also activates telomerase and stabilises active Src-family kinases involved in enhanced cell survival, proliferation, and motility. The E7 Protein binds to and degrades the Retinoblastoma (Rb) protein (10). The RB gene, initially identified as the gene responsible for childhood eye tumours, was one of the first tumour suppressor genes to be discovered and led to Knudson's famous "two-hit" hypothesis of cancer development (11).

1.2. HPV Genotype and Neoplastic Progression

Some HPV genotypes are high-risk (HR) for inducing squamous and adenosquamous cervical cancers, while others are only capable of producing genital warts or low grade dysplastic lesions(5). A DNA test for thirteen HPV-genotypes (HPV-16,-18,-31,-33,-35,-39,-45,-51,-52,-56,-58,-59,-68) as been approved (12).

Particular HPV genotypes (e.g. HPV-31,-32,-52,- 58) allow neoplastic progression to CIN3 only, whereas others (e.g HPV-16,-18 and -45) preferentially progress from CIN3 to cancer (13). Dysregulated expression of E6/E7 proteins leads to increased cell proliferation in the lower epithelial layers coupled with an inability to repair mutations in the host DNA. Integration and high expression of viral E7 genes within host DNA is a feature of progression from CIN3 to invasive cancer, as are loss of E2 and E4 genes which can exert a negative growth effect.14

1.3. The HPV life cycle and associated genetic events

The HPV life cycle consists of initial infection, uncoating, genome maintenance, genome amplification, and packaging to form new viral particles. The viral E1 and E2 proteins have a role in maintaining the viral genome as an independent DNA element which can replicate extra-chromosomally or can be maintained by integration into the host genome (episome). Normal basal epithelial cells undergo cell division but then exit the cell cycle as they reach the suprabasal layers and undergo a process of terminal differentiation. In HPV infected cells the viral E6 and E7 proteins are expressed which prevent the suprabasal cells from exiting the cell cycle and retard the differentiation process (14). Excessive amounts of p21/p27 can bind E7 and reduce the proliferative effect. It has therefore been suggested that E7 functions to promote S-phase entry in a subset of suprabasal cells with intrinsically low levels of p21/p27 or alternatively high levels of E7 expression(14).

Amplification of viral genome occurs in the mid to upper epithelial layers and requires the activity of E1, E1, E4 and E5 proteins. The exact details are still to be elucidated but key events include up-regulation of a promoter present within the E7 open reading frame and increased E1/E2 expression. The freshly replicated genome can act as a template for further gene expression leading top increased amounts of E1/E2 and other replication proteins. Interestingly, the virus is non-lytic and remains within the epithelial cell until the latter reaches the epithelial surface. Our study mainly focused to HPV detection and HPV genotyping from paraffin embedded tissues and cervical scrapings from women with cervical carcinoma.

II. Methodology

Samples and DNA Isolation: Our study includes 50 samples of cervical carcinoma and 50 samples of age and gender matched control population. Samples were obtained from STD OP and Gynaec OP of King George Hospital, Gynaec OP of Victoria General Hospital and Mahatma Gandhi Cancer Hospital, Visakhapatnam in the period of December 2011 to December 2013. Cervical scrapings and paraffin embedded tissue (PET) were collected from each histopathologically positive for cervical cancer patients and were stored at -20° C. The DNA was isolated by a simple method (**Santos et al., 2008**) (21 & 22).

Amplification: A polymerase chain reaction (PCR) was then carried out to amplify the DNA sequences of interest by denaturing the DNA molecule and replicating it by utilizing primers, free nucleotides, and a polymerase designed to help the DNA withstand the high temperatures involved in PCR. Because PCR can only be applied when the nucleotide sequence of at least one DNA segment is known, primers were used. The HPV forward primer was 5'- TTTGTTACTGTGGTAGATACTAC -3'and its reverse primer was 5'-GAAAAATAAACTGTAAATCATATTC -3'. HPV16 forward primer was 5'- TACCTACGACATGGGGAG -3'and its reverse primer was 5'- TGACAAGCAATTGCCTGGGT- 3, HPV18 forward primer was 5'-CCTGGGCAATATGATGCTA -3'and its reverse primer was 5'- CCTTATTTTCAGCCGGTGCA- 3''. PCR conditions for HPV are one minute denaturing at 95°C, 30 seconds annealing at 55°C, and 1 minutes extension at 72°C for 40 cycleS, for HPV16, one minute denaturing at 95°C, three minutes annealing at 58°C, and 1 minutes extension at 72°C for 40 cycles and for HPV18, one minute denaturing at 95°C, three minutes annealing at 60°C, and 1 minutes extension at 72°C for 40 cycles.

Electrophoresis and Analysis: Agarose gel electrophoresis was then performed to analyze the DNA samples using a 2% agarose gel at 100 V for 30 minutes. Ethedium bromide was used to colour each band under ultraviolet light. Band sizes were subsequently compared to the molecular weight band markers for 100-1000 base pairs for confirmation.

III. Results

Fig 1: shows cervical cancer patients HPV PCR Product.

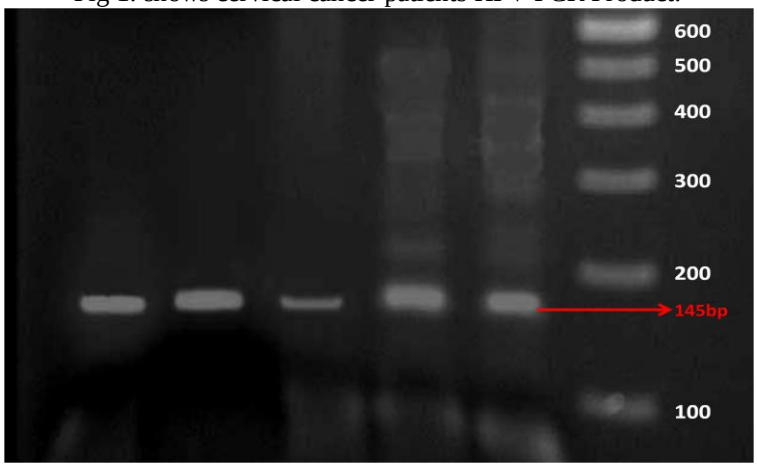


Fig -2: shows the positive bands for HPV 16

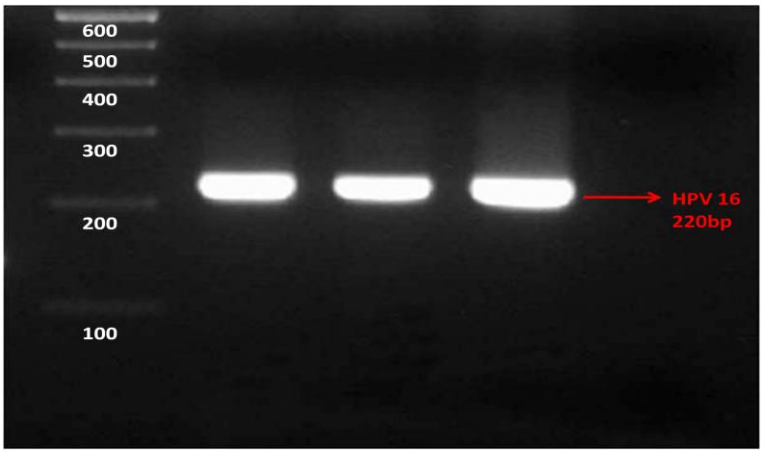
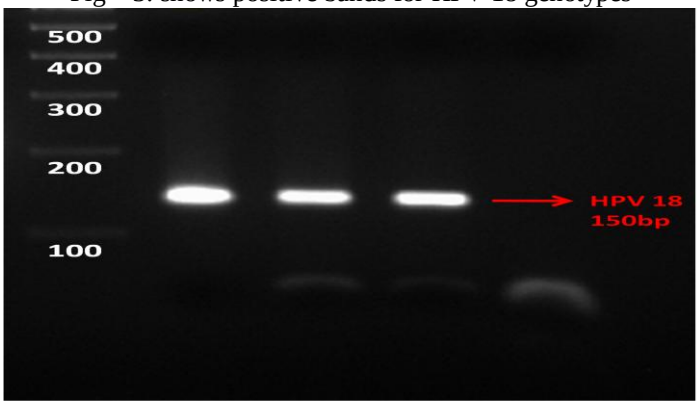


Fig – 3: shows positive bands for HPV 18 genotypes



HPV 16 is the most commonest type in cervical cancer as well as general population. High prevalence of cervical cancer we see in age group of 29 -50 (Fig-4).

Fig – 4: shows HPV DNA prevalence in women with Cervical Cancer belonging to different age strata

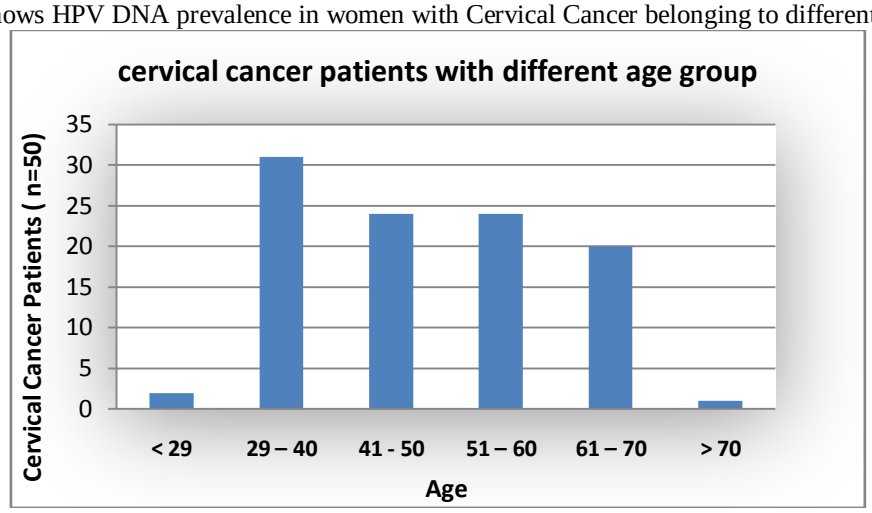
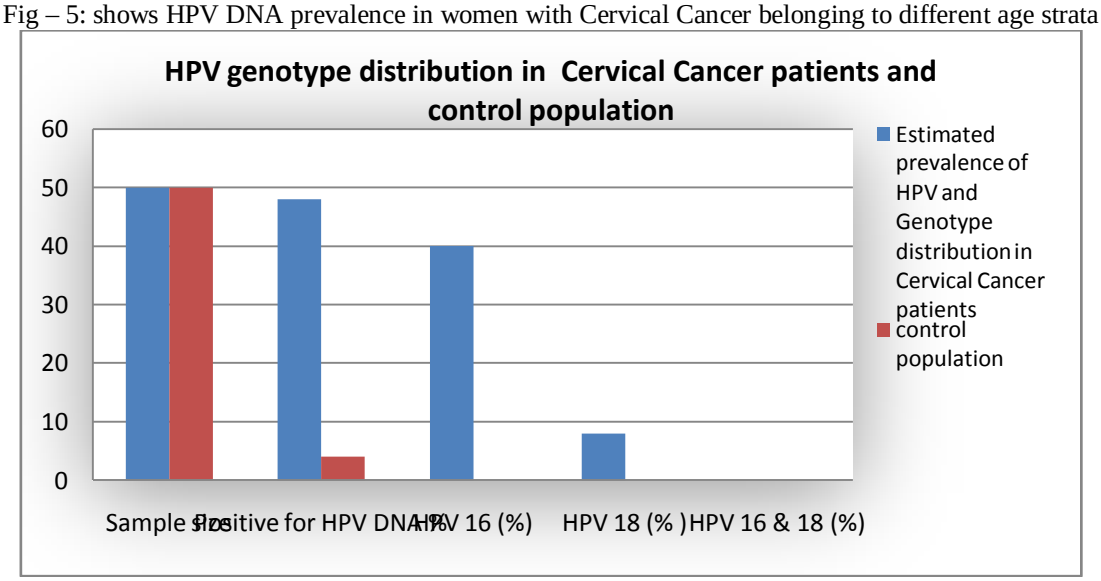


Fig – 5: shows HPV DNA prevalence in women with Cervical Cancer belonging to different age strata



HPV was identified in 48% of patients with cervical cancer and 4% of controls. The present study showed the presence of HPV type 16 in 40 patients (80%) where as HPV type 18 in 08 patients (16%) with cervical carcinoma.

IV. Discussion

The purpose of this study was to investigate the presence of HPV genotypes in cervical cancer patients using the polymerase chain reaction (PCR). Extracted DNA was evaluated for HPV infections by PCR analysis using consensus primers specific for HPV (HPVF, HPVR), HPV 16 and HPV 18. Out of 50 samples, 48 samples were positive for HPV, 40 samples were positive for HPV16 genotype and 08 samples were positive for HPV18 genotype and control population was HPV positive in 4% of population. There was statistically significant association of HPV 16 with cervical carcinoma or HPV infection. Most of the work in this area and its associated genetic events has focused on HPV type 16 (HPV16) which is a major cause of cervical cancer. Initial infection is thought to require viral access to cells in the basal layer of the epithelium, via breaks, abrasions or other micro-traumas in the stratified epithelium. Hair follicles seem to have abundant amounts of viral DNA (15) leading to suggestions that epithelial stem cells may be an important target for the virus (16). The virus attaches to the basal epithelial cells via specific cell surface receptors (17) leading to internalisation of the virus followed by uncoating of the viral particles and release of the viral genome. Clinically, HPV-associated carcinoma was present in younger patients in comparison with age (mean age, 48.6 versus 56 years; P = 0.001). The odds for patients with HPV infection to develop carcinoma were 20 times greater than for patients without HPV infection (95% confidence interval 4.1, 74.2) (21). We investigated the HPV type distribution in invasive cervical cancer. Different HPV types are associated with different cancer risks (24), and circulation of HPV types among women without cancer may therefore not accurately reflect which HPV types cause invasive cervical cancer in a population.

V. Conclusion

High-risk HPV types 16 and 18 are together responsible for over 96% of cervical cancer cases. Type 16 causes 54 to 80% of cervical cancers, and accounts for an even greater majority of HPV-induced vaginal/vulvar cancers, penile cancers, anal cancers and head and neck cancers. Use of multiple specimen types or the development of highly sensitive and robust in situ hybridization HPV-testing methods to evaluate the certainty of attribution of lesions to HPV types might provide insights in future efforts, including HPV vaccine trials.

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