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Prevalence Of Hpv 16 And 18 Genotypes In Cervical Biopsies From Women Diagnosed With Cervical Cancer In Visakhapatnam, Andhra Pradesh.

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Abstract
Background: *Cervical cancer is a cancer of the cervix, the fourth deadliest cancer in women. Diagnosed in more than 90% of cervical cancers, Human papilloma virus (HPV) is currently the most common pathogen responsible for female cancers. Numerous molecular genetic studies have provided strong evidence that HPV is an oncogenic virus. HPV was found to inactivating some of the mechanisms which regulating the cellular mitotic cycle. Due to this mitotic insult, the virus takes an advantage to launches a cascade of uncontrolled genetic events that may lead to malignant transformation of the host cell.*
Purpose: *The main objective of the study was to determine the association between HPV infection and cervical cancer and to investigate age wise distribution of HPV genotypes 16 and 18.*
Methods: *The study includes more than 100 (115) specimens in the period of November 2022to December 2023 from patients with invasive cervical cancer were collected from King George Hospital, Andhra Medical College and some private hospitals in and around Visakhapatnam, Andhra Pradesh. We used polymerase chain reaction (PCR) assay capable of detecting HPV 16/18 genotypes.*
Results: *HPV DNA was detected in 98% of the cervical biopsies, with no significant variation in HPV positivity. HPV 16 was present in 93% of the specimens, HPV 18 in 5.3%. The Chi-square test was used to compare the differences in HPV genotype distribution among the histopathologically different lesions and all age (20-64 yrs) groups. The chi-square statistic is 10.0797 (N=115). The p-value is 0.039. The result is significant at p-value <0.05.*
Conclusions: *This study explores the epidemiological data of the prevalence and genotype distribution of HPV in patients with CIN in costal Andhra Pradesh, Visakhapatnam population. We found that different age groups have different HPV genotype distributions in cases of CIN.*
Keywords: *Cervical cancer, cervical intraepithelial neoplasia (CIN), HPV, genotypes, DNA, PCR.*

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I. Introduction

The cervix is the part of the uterus that connects to the upper part of the uterus and vegina and it plays a major role in the menstrual cycle, fertility, pregnancy and child birth. Cervical cancer is the fourth most common cause of cancer deaths after breast, colorectal and lung cancers in women globally [1]. Prolonged infection with certain types of human papillomavirus (HPV) that transmit during sex is the main cause of cervical cancer [2]. When a woman becomes infected with certain high-risk types of human papilloma virus HR-HPV and does not clear the infection, abnormal cells can develop in the lining of the cervix or precancerous intraepithelial lesions and is one of the most common sexually transmitted infections worldwide. If not discovered early and treated, these abnormal cells can become cervical precancers and then possibly cancer. Most of the cases it can take a number of years, although in rare cases it can happen within a year. HPV is a common oncovirus that affects both females and males [3]. Most types of HPV are harmless, do not cause any infections and malignancy, and go away on their own. There are about 30 types of HPV are known to considered as genital HPV since they affect the genital area that are benign (noncancerous) [4-7]. Few types are high risk and they may lead cervical cancer or abnormal cells in the lining of the cervix that sometimes leads into cervical cancer and cervical intraepithelial neoplasia (CIN) [8]. Due to this clear relationship between HPV and cervical cancer, is an avoidable disease that can be prevented, treated and eradicated, compared to many other cancers. According to infection rate of epithelial tissue, CIN can be graded on 1–3 scale, where CIN3 is the most abnormal grade [9]. In this study, patients who had undergone HPV examination and cervical pathological biopsy in the King George Hospital, Andhra Medical College and some private hospitals in and

around Visakhapatnam, Andhra Pradesh, from June 2019 to June 2023 were recruited, HPV genotypes were identified, and the distribution of HPV types in different age groups was screened and analysed.

II. Materials And Methods:

Study Population

The present study was conducted among married women of reproductive age (20–64 years) women diagnosed with cervical intraepithelial neoplasia or cervical cancer, residing in and around Visakhapatnam Andhra Pradesh, India.

Exclusion Criteria

The patients who were excluded from this study were pregnancy, severe gynaecological problems, hysterectomy or previous history of the disease.

Ethical Approval

This study has been approved by the Institutional Ethics Committee (IEC) of the Andhra Medical College, Visakhapatnam.

Sampling and Sample Size

115 known cervical cancer biopsy samples and few (n=4) paraffin embedded tissues (PET) samples were recruited in the present study in the period of November 2022 to December 2023. From the blocks 10µ sections were taken and inserted in 1.5ml micro centrifuge tubes. They were subjected to various extraction methods which are described below. PET samples were underwent deparaffinization with xylene, followed by rehydration in graded ethanol. Subsequently, DNA was extracted from each sample using the QiaAmp mini nucleic acid extraction kit.

PCR technique is a method for the detection of particular desired gene, in this study we used for the detection of high-risk forms HPV of which are responsible for cervical cancer. The quality of the DNA was evaluated using PCR amplification of β-globin gene the housekeeping gene as an internal control. HPV-positive samples were confirmed by PCR using consensus primers (GP5/GP6) [10-11]. The PCR with primers GP5 and GP6 in reaction volume of 20 µl was setup. The final concentrations of all the reagents were as follows. GP5: 5'-TTT GTT ACT GTG GTA GAT ACT AC-3' and GP6: 5'- GAA AAA TAA ACT GTA AAT CAT ATT C-3', The 268 bp fragment of the β-globin gene amplification was used to assess the quality of DNA in samples. The primers were GH20 (5' GAA GAG CCA AGG ACA GGT AC3') and PC04 (5'-CAA CTT CAT CCA CGT TCA CC-3', 50 mMolar KCl, 10 mMolar Tris, 200 µMolar dNTP's, 1.5 mMolar $MgCl_2$, 4 units of taq DNA Polymerase and 20 pMolar of each primer. The PCR reactions were setup under cycling parameters of denaturation at 94^o C for 4 minutes, 94^o C for 30 seconds, 55^o C for 30 seconds, 72^o C for 30 seconds and the final extension was of 72^o C for 10 minutes. The number of PCR thermal cycles were used 45.

PCR for the detection of HPV 16 & 18 genotypes was performed using specific primers HPV E6/E7 gene of HPV 16 and 18 using type-specific primers (genotype-specific PCR). The primer sequences were HPV16 E6: forward 5'-CTG CAA GCA ACA GTT ACT GCG ACG-3', reverse 5'-CAT ACA TCG ACC GGT CCA CC-3', product of 315 bp; HPV 18 E7: forward 5'-GAG CCG AAC CAC AAC GTC AC-3', reverse 5'-GGA TGC ACA CCA CGG ACA CA-3', product of 152 bp [12]. HPV genotyping was completed using the HPV biorad kit, following the manufacturer's guidelines. PCR was performed using a reaction volume of 25µl, containing 5 µl of DNA template, 19.25 µl of the provided master mix, and 0.5µl DNA Taq polymerase, using a CFX96 (Bio-Rad). The amplification procedure was per-formed as follows: 9 min of denaturation at 95◦C, followed by 40cycles of 20 s of denaturation at 95◦C, 30 s of annealing at 55◦C, 30 seconds of elongation at 72◦C, and a final extension for 5 min at 72◦C.

Statistical analysis

The mean age of our study population is 39.8yrs. The Chi-square test was used to compare the differences in HPV genotype distribution among the histopathologically different lesions and all age (20-64 yrs) groups. The chi-square statistic is 10.0797 (1, N=115). The p-value is .039. The result is significant at p-value <.05.

	Age <35 (n = 25)	**35-44 (n = 64)**	**45-54 (n = 21)**	**55-64 (n = 05)**
HPV16	24	61	19	4
HPV18	1	2	2	1
Co- infection	-	1	-	-

Table:1 shows the HPV genotype distribution among the patients with different age groups

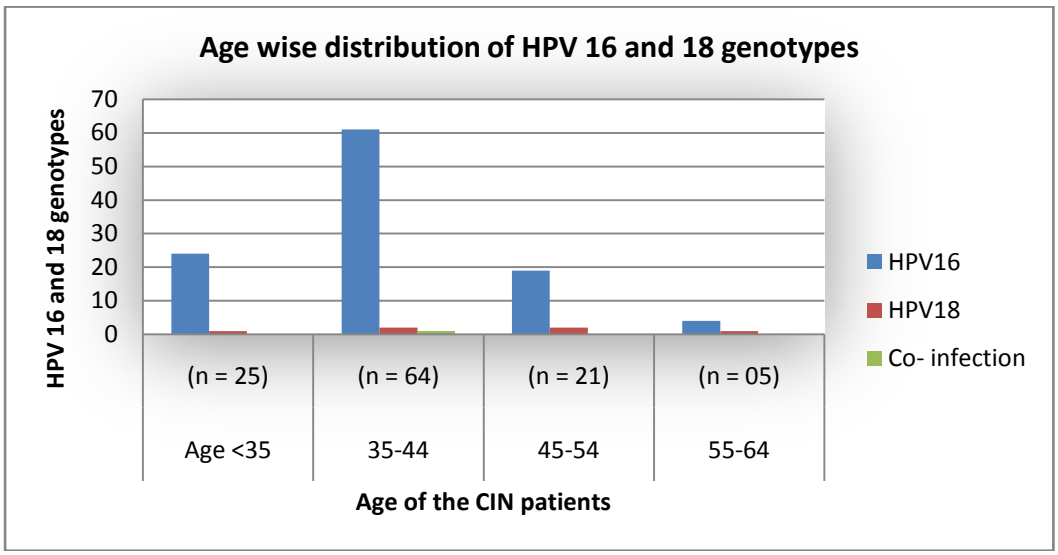

Figure- 1: Distribution of HPV 16 and 18 genotype in patients with cervical cancer.

N=115 samples of cervical biopsies which are confirmed cervical cancer tissues were analyzed. In n=108 samples gave positive for HPV genotype 16 i.e. around 93% and n=6 samples gave positive for HPV genotype 18 (5.2%) n=1 gave positive for both HPV genotypes 16 and 18 (Co-infection) (0.8%) of the cervical cancer patient are infected with HPV.

III. Discussion

A diagnostic view-point, the consistent presence of Human Papilloma Virus in Cervical Cancer allows the viral DNA to be used as a genetic marker [12-13]. Numerous molecular genetic studies have been provided strong evidence that HPV is an oncogenic virus [13]. HPV was found to inactivating some of the mechanisms which regulate the cellular mitotic cycle. Due to this mitotic insult, the virus takes an advantage to launches a cascade of uncontrolled genetic events that may lead to malignant transformation of the host cell [14]. The fidelity of cellular DNA replication is regulated by tumour suppressor gene, called p53. A potential change of p53 gene expression by HPV renders cellular DNA susceptible to carcinogenic effects of mutagens (Ex: carcinogens from nicotine products) [15]. High oncogenic risk HPVs have been detected in virtually 100% of carcinomas of the uterine cervix and the role of HPV in malignant transformation of the cervical epithelium has been well established [16-17].

In this study, we also found differences in the prevalence and genotype distribution of HPV between different age groups. The differences found between the age groups could be attributable to the fact that HPV prevalence and genotype distribution vary significantly among different age groups [18]. The distribution of HPV genotypes in women with cervical cancer significantly varies across populations and geographic regions of the world, and these differences might be correlated with complex geographical variations and biological inter play in different HPV genotypes and host immunogenic factors [19]. Human Papilloma Virus genotypes 16 & 18 are the most common genotypes throughout the world. These findings are correlated with those of previous studies. It is well established that HPV-16 is the most commonly encountered HPV genotype in the world, whereas the frequencies of the other genotypes vary geo-graphically [20]. However, assessment of HR-HPVs in the pathogenesis of cervical cancer in and around Visakhapatnam is essential in order to evaluate the status for future plan of prevention strategies including vaccination against HPVs. In this study, we present an additional evidence of the existing epidemiological evidences regarding the presence of HPV in cervical cancers in Visakhapatnam and the potential need for vaccination against HR-HPV infections, particularly HR-HPV 16 and 18 and its effect on human health in state of Andhra Pradesh. Co-infections of both HR-HPV 16 & 18 types were identified in n=1 (0.8%) of the patients. This percentage is much lower than reports from highly HPV endemic areas which ranging from 30% to 65% (21).

The genotype assay that was used in this study, did not completely genotype all HPVs. Due to the minimal sample size and selected parameters; the results generated from this study are limited and cannot be generalized. Further research suggested to clarifying these findings in the future.

IV. Conclusion

In conclusions, the rate of infection with HR-HPV 16 and 18 is relatively higher in and around Visakhapatnam with predominance of HR-HPV type 16. This study may provide valuable data for future

evaluations, management including; prevention, treatment of HPV infection, cervical cancer and HPV vaccinations.

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Conflicts of interest
No conflicts of interest.

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