Selected Publications

1. **Rajeev Kumar,** Sankar Kumar Ghosh, Akalesh Kumar Verma, Anuradha Talukdar, Monoj Kumar Deka, Iqbal Bahar, Mira Wagh, Ritesh Tapkire, Kali Pankaj Chakarborty, Ravi Kannan. p16 expression can predict response to neo adjuvant chemotherapy in esophageal squamous cell carcinoma patients. APJCP, VOLUME 16, 2015 **Issue Number 16 ,** 7161-7165

2. Human Papillomavirus Testing for Suspected Cervical Cancer Patients from Southern Assam by Fast- PCR.SK Ghosh, B Choudhury, J Hansa, A Das, **R Kumar,** R S Laskar, R Kannan, P R Ghosh, 2011, Asian Pacific J Cancer Prev, 12, 749-751.

3. **Rajeev Kumar,** R Ravi Kannan, Sankar K. Ghosh, Sumit Goyal, Jagadish Hansa, Aditi Bhowmik. (2011): Relationship between various Biochemical, Haematological, Co-morbid, Life-style, Demographic parameters in Gallbladder Carcinoma: A single centre retrospective study of 342 patients.), American Association for Cancer Research, 260375_2.

4. **Rajeev Kumar,** Sankar Kumar Ghosh, Akalesh Kumar Verma, Anuradha Talukdar, Monoj Kumar Deka, Iqbal Bahar, Ritesh Tapkire, Ravi Kannan. ALDH1 predicts non responders among esophageal squamous cell carcinoma patients who undergone neo-adjuvant chemotherapy. (Under review)

RESEARCH ARTICLE

p16 Expression as a Surrogate Marker for HPV Infection in Esophageal Squamous Cell Carcinoma can Predict Response to Neo-Adjuvant Chemotherapy

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Abstract

Background: Esophageal squamous cell carcinoma (ESCC) is a common cancer in the north east of India. The present study concerned the prevalence of human papilloma virus (HPV) in the ESCC in north eastern India and its impact on response to chemotherapy. Materials and Methods: p16 expression, a surrogate marker for HPV infection was assessed in 101 pre-treatment biopsies of locally advanced ESCC, reported from a comprehensive cancer centre in north east India, using immunohistochemistry. All patients received neo-adjuvant chemotherapy. Response was assessed clinically and histopathologically with attention to p16 expression. Results: p16 was expressed in 22% of ESCC (22 out of 101) and was more prevalent in patients who were more than 45 years of age (P=0.048). p16 positive tumors appeared more commonly in the upper 2/3 of the thoracic esophagus (18 in 22). Nine of the 22 (41%) p16 positive tumors achieved pathologic complete response following neo-adjuvant chemotherapy (P=0.008). There was a trend towards reduced mortality in this group (P=0.048). Some 9 of the 20 (45%) patients who achieved pathologic complete response were p16 positive. Conclusions: Expression of p16 in ESCC correlates with higher rate of pathologic complete remission in patients undergoing neo adjuvant chemotherapy and could be a predictive marker for response assessment.

Keywords: p16 - HPV - esophageal squamous cell carcinoma - response assessment - neo adjuvant chemotherapy

Asian Pac J Cancer Prev, **16 (16)**, 7161-7165

Introduction

Several reported epidemiological studies worldwide have identified the high risk countries for esophageal cancer that included the People's Republic of China, Singapore, former USSR, Iran, Puerto Rico, Chile, Brazil, Switzerland, France, and South Africa (Syrjanen, 2002). It is the eighth most common cancer worldwide, with an estimated 456,000 new cases in 2012, and the sixth most common cause of cancer related death with an estimated 400,000 deaths (IARC, 2014). India is among those Asian countries that shares the high burden of esophageal cancer (Chung et al., 2010). North eastern India falls into region adjoining southern edge of the Asian esophageal cancer belt.

The age adjusted incidence rates (AARs) of 7.6 and 5.1 per 1,00,000 of esophageal cancer were observed in India as compared to USA (4.9 and 1.4) for males and females, respectively (Ali et al., 2011). In north eastern India, where the tobacco and areca nut use is rampant, incidence of ESCC is relatively high. The age adjusted incidence rates of esophageal cancer per 100,000 persons for males and females from the high-risk region of Northeast India have been 71.4 in males and 30.2 in females from East Khasi Hills of Meghalaya state as reported by National Cancer Registry Program (NCRP), Indian Council of Medical Research (ICMR, NCRP 2013). Population based cancer registries in other parts of north east India reported higher incidence of esophageal cancer. The AARs observed in Meghalaya, Kamrup Urban district and Cachar district are 46.2, 27 and 11.7 in males whereas 19.8, 18.3 and 8.3 in females respectively (NCRP 2013).

Esophageal cancer comprises of two main histopathology types, adenocarcinoma and squamous cell carcinoma. Both types have their specific characteristics in terms of etiology, epidemiology, molecular, therapeutic and prognostic aspects. Esophageal adenocarcinoma (EA) is associated with gastro-esophageal reflux and obesity, whereas esophageal squamous cell carcinoma (ESCC) is associated with use of tobacco and alcohol (Lagergren and Lagergren 2010). ESCC is more common with an approximate percentage of 80% than EA (20%) in India (Pramesh et al., 2015).

Syrjanen in 1982 had reported human papillomavirus

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(HPV) infection as one of the possible etiological factors in ESCC. However HPV positivity using immunohistochemistry in ESCC is conflicting that ranges between 10 to 40% of HPV in tumor tissues (Syrjanen, 2002; Michaelsen et al., 2014).

p16 expression is usually used as a surrogate marker for detecting HPV infection due to its high sensitivity, low cost and simplicity (Kalof and Cooper, 2006; Naggar and Westra, 2012).

In India, the incidence of esophageal carcinoma differs from region to region and the association of HPV infection in ESCC has been demonstrated in some of studies (Shukla et al., 2009; Mohiuddin et al., 2013; Vaiphei et al., 2013).

HPV infection has been reported to correlate with the clinical outcome in different squamous cell malignancies. p16 expression that is correlated with HPV infection was shown as marker for responder and better prognosis among head and neck squamous cell carcinoma patients who underwent radiotherapy (Lassen et al., 2009). Chemotherapy could be omitted in this group of patients. Similarly there is some evidence to suggest that high p16 expression correlates with favourable prognosis in esophageal squamous cell carcinoma as well (Sturm et al., 2001; Cao et al., 2014).

There exist limited and inconsistent reports on the relationship between p16 expression and response to neo adjuvant chemotherapy that consists of standard platin, taxan and 5 fluro uracil based agent in ESCC patients particularly from north east India. This study intended to find out prevalence of HPV infection as correlated by p16 expression and its impact on response to neo adjuvant chemotherapy in ESCC.

Materials and Methods

Patient Selection: This was a retrospective study involving 101 consecutive ESCC patients who reported at the Cachar Cancer Hospital Research Centre, Silchar, India from 2010-14. All of them presented with locally advanced esophageal squamous cell carcinoma and majority were in performance status 1 during initial presentation. They were therefore advised neo adjuvant chemotherapy. The chemotherapy was as per national comprehensive cancer network (NCCN) guidelines that comprised of platin, taxan and 5 fluro uracil. Institutional Review Board of Cachar Cancer Hospital & Research Centre, Silchar, India approved (IRB/CCHRC/07/2013) this study. Formalin fixed paraffin embedded tissue blocks (FFPE) along with clinical information were used as per approved guidelines. Endoscopies and Computed Tomography were employed to stage the disease status. Endoscopic guided punch biopsies were taken for histopathology reporting and immunohistochemistry (IHC) tests.

Patients habit and medical data was extracted from the respective patient's record. Performance status of patient on first visit was assessed on Easter Cooperative Oncology Group (ECOG) scale (Oken et al., 1982).

Immunohistochemistry: Glass slide treated with 3-Aminopropyltriethoxysilane (Sigma-Aldrich) was used for adhering 4 μm tissue cut section from paraffin blocks. It was left for drying under fan for 1 hour and subsequently inside incubator at 50°C for subsequent 12 hours or overnight. Positive controls (cervical cancer) and negative control (without primary antibody) were used during each cycle of experimentation. Slides were heated at 60°C for 1 hour that was followed immediately with 2 step xylene (Merck) wash, 10 minutes each for deparaffinization. Thereafter, it was rehydrated with graded alcohol for a total of 20 minutes and was kept for 1 minute in distilled water. Antigen retrieval was performed by placing slides in pre-warned (65°C) Tris-EDTA (pH 9; Dako) buffer and heating it at 97°C for 20 minutes with 400 watt in microwave oven. After cooling it to room temperature, blockage of endogenous peroxidase was performed by immersion in 3% hydrogen peroxide (Dako) for 10 minutes. After 5 minutes wash with phosphate buffered saline (PBS), sections were incubated with primary monoclonal antibody: $p16^{INK4a}$ (Clone JC8; delusion 1:150; Santa Cruz Biotechnology, USA) in humidified chamber for 1 hour. Excess primary antibody was washed with PBS. Envision Flex/HRP (Dako) was applied as secondary antibody over slides and incubated for 30 minutes in humidified chamber. After washing slides with PBS, 3,3-diaminobenzidine (Dako) was applied as chromogen for 10 minutes. Finally, sections were washed with distilled water, counterstained with haematoxyline for 45 seconds, rehydrated with graded alcohol, dried and mounted with DPX for analysis.

Immunohistochemical scoring: p16 IHC scoring was done by 2 independent pathologists blinded to this study. Strong and uniform p16 staining (both cytoplasmic and nuclear) in all or most cancer cells were considered p16 positive whereas absent or weak p16 staining in cancer cells were considered as negative (El-Naggar and Westra, 2012). Sections which shows strong p16 expression in more than 50% for tumor cells was considered as positive and less than 50% expression was considered as negative for statistical analysis.

Response assessment: Following neo adjuvant chemotherapy (NACT), patient underwent surgery. Response was assessed based on the histopathology of the resected specimen. For patients who received concurrent chemo radiotherapy following NACT, response was assessed by endoscopy and computed tomography scan. Patients who achieved complete remission were considered responder. Patients with any residue were grouped as non responders.

Statistical method: Statistical analysis was performed by using χ^2 with likelihood ratio (LR) or Fisher's exact test for correlation analysis. Software SPSS version 10 was employed for statistical analysis. In all tests, 2 tail P value was utilized. Test was considered statistically significant when $P \leq 0.05$.

Results

Correlation analysis of clinicopathological factors with p16 expression in ESCC patients is shown in Tables 1 and 2. 94% of patients who were reported in this study were from Barak valley in Southern Assam and were from Bengali speaking community. Over expression of p16 was reported in 22% of ESCC (22 out of 101) patients. Those

who had shown p16 over expression, majority (91%) were more than 45 years old (P=0.048). 59% of p16 positive patients were males (13 out of 22) (P=0.421). 97% ESCC patients presented with dysphagia as an initial symptom. From that 22% (21 out of 97) tested positive for p16. Initial Eastern Cooperative Oncology Group (ECOG) performance status was reported either 0 (normal) or 1 (near normal) or 2 (difficulty in normal activity) in 17%, 66% and 16% patients respectively. p16 positive tumors appeared more prominently in upper two third of thoracic region (82%) and tended to expressed more in moderately differentiated ESCC (59%). However, ESCC tumors that were found positive for p16 expression appeared to fall into responders group rather than non responders (P=0.008) and reported with less mortality (P=0.048). 41% p16 positive were found responders whereas 45% responders were observed p16 positive.

Table 1. Correlation analysis of clinicopathological factors with p16 expression in ESCC patients

	p16 Positive	p16 Negative	P-value
Demography			0.314
Barak Valley	20	74	
Halflong	1	2	
Tinsukia	0	1	
Kamrup	1	0	
Tripura	0	2	
Age (in Years)			0.048
≤45	2	22	
>45	20	57	
Gender			0.421
Male	13	54	
Female	9	25	
Initial symptom			0.21
Dysphagia	21	76	
Pain in abdomen	0	1	
Dysphagia with hoarseness of voice	0	2	
Pain in supraclavicular	1	0	
ECOG Performance status (Initial presentation)			0.152
0	1	16	
1	16	50	
2	5	11	
3	0	2	
4	0	0	
Cancer Site			0.48
Cervical	0	1	
Upper thoracic	6	11	
Mid thoracic	12	52	
Lower thoracic	4	15	
Grade			0.435
Well differentiated	7	19	
Moderately differentiated	13	57	
Poorly differentiated	2	3	
Neo adjuvant chemotherapy			0.008
Responders	9	11	
Non Responders	13	68	
Death			0.048
Yes	6	40	
No	16	339	

Abbreviations: ECOG = Eastern Cooperative Oncology Group

Table 2. Correlation Analysis of Reported Habits with p16 Expression in ESCC Patients

	p16 Positive	p16 Negative	P-value
Cigarette smoking			0.868
Yes	11	42	
No	8	28	
Tobacco chewers			0.313
Yes	6	31	
No	13	39	
Alcohol intake			0.417
Yes	3	17	
No	16	53	
Areca Nut			0.485
Yes	15	60	
No	4	10	
Pan			0.297
Yes	13	56	
No	6	14	

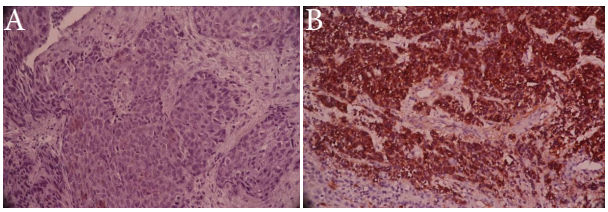

Figure 1. p16 Expression in ESCC. A) p16 negative ESCC tumor; B) p16 positive ESCC tumor

p16 positive staining was observed in 22 cases as represented in Figure 1. Information related to habit was available for only 89 ESCC patients. Out of which 21% were positive for p16 and remaining 79% were negative. Predominantly 84% were reported using areca nut, 60% smokers, 42% tobacco chewers, 77% pan users and merely 22% remarked alcohol intake.

Discussion

Our understanding of the role of HPV in carcinogenesis is evolving. It is strongly implicated as a causative factor in the tumorogenesis of squamous cell carcinoma of cervix (Walboomers et al., 1999; Hausen, 2009). There is a strong association of HPV with squamous cell cancer of the head and neck, commonly oropharynx (D'Souza et al., 2007), where p16 expression act as predictive marker for

response to radiation therapy. Its role in ESCC (Liyanage et al., 2013) is unclear. However, it is implicated in some studies as predictive and prognostic marker of squamous cell carcinomas (Lassen et al., 2009; Fischer et al., 2010; Cao et al., 2014).

Prevalence of HPV as evidenced by p16 over expression in ESCC was found in 22% (22 out of 101) of studied cases in our study. It correlates with the results of a recent meta analysis of 13832 ESCC patients involving 124 studies, where the average prevalence of HPV was found 0.304 (0.185, 0.423) by immunohistochemistry with 95% confidence interval (Petrick et al., 2014). A similar meta analysis based on 132 studies related to HPV in ESCC by Hardefeldt HA found HPV prevalence in ESCC at 24.8% (Hardefeldt et al., 2014).

However there exists inconclusive evidence of its aetiological significance in the development of ESCC (Mohiuddin et al., 2013; Zhang et al., 2014). Few Meta studies though have reported that HPV infection can increase the risk of developing ESCC by 3 folds (Liyanage et al., 2013).

In this study male ESCC patients were reported approximately two times more than their female counterpart with a male to female ratio of 1.97:1. This is similar to literature report of male to female ratio of 1.8:1 (Mohiuddin et al., 2013). p16 expression was however more common in ESCC in women's. We reported 26% of ESCC in females and 19% in males overexpressed p16 (P=0.421) in this study.

This study reported 69% ESCC tumors were in moderately differentiated grade where 19% were shown p16 positivity. In squamous cell carcinoma of cervix, p16 positivity has been reported in 100% of patients (Ma et al., 2010). We found HPV infection was more prevalent in upper thoracic region (35%) than mid (19%) and lower thoracic region (21%) that may be due to its proximity to oropharyngeal squamous epithelium, which acts as route for HPV infection. Similar result was also reported by Fangli et al., 2014.

We found 43% those with pathologically complete response were p16 positive whereas 16% non responders positive for p16. In this study we have found p16 expression more common in responders (P=0.009), which indicates that p16 correlated HPV positive tumors show favourable outcome in terms of reaching pathologically complete response (PCR) of tumor after the complete regime of neo adjuvant chemotherapy. Patients who shown p16 positivity were reported less deaths (27%) and among all reported deaths, majority 87% were p16 negative (P=0.048). This correlates with similar finding where HPV positivity was shown to increase progression free survival and disease free survival in patients with squamous cell carcinomas (Ang et al., 2010; Fischer et al., 2010; Fangli et al., 2014).

The role of tobacco and areca nut chewing, tobacco smoking and alcohol drinking have shown to be a risk factor in the tumorogenesis of different types of squamous cell carcinoma including ESCC (Lee et al., 2005; Ko et al., 1995; Muwonge et al., 2008; Lagergren and Lagergren 2010; Pramesh et al., 2015). Although the prevalence of areca nut (84%), pan (77%), cigarette (60%), tobacco using (42%), were high in ESCC patients, we found no statistical significance of these factors with p16 expression. Extensive use of areca nut and tobacco in this region corresponds to the finding of highest esophageal cancer AAR of 71.4 in males and 30.2 in females reported in East Khasi Hills of Meghalaya state in north east India whereas in Asia it reported second highest in China, Jiashan at AAR 20.2 for males and in Pakistan, South Karachi at AAR 8.6 for females (NCRP 2013).

The percentage of alcohol use among ESCC patients was found considerable low at 22% in our study. It show no correlation with the available report where alcohol was considered as one of the important risk factor for developing ESCC (Blot, 1999; Lagergren and Lagergren 2010).

Current data though suggests the role of p16 as predictive marker for treatment response assessment however a similar study on a larger number of ESCC patients will provide further evidence that is required to implement this finding in routine clinical settings for better management of ESCC.

Acknowledgements

DBT (Gov. of India) for funding.

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RESEARCH COMMUNICATION

Human Papillomavirus Testing for Suspected Cervical Cancer Patients from Southern Assam by Fast-PCR

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Abstract

World-wide epidemiological studies have shown that cancer of the uterine cervix is the second most common malignant disease in women. Virtually every cervical cancer (99.7%) is HPV-positive, indicating that the presence of HPV is an obligatory element in their development. The present study was conducted by Fast-PCR (within 15 min.) based diagnosis of HPV 16 and HPV 18 infection amongst patients of suspected cervical cancer, confirmed by cytological methods. Twelve women, out of a total of fifty studied cases who had positive cervical pap smears (24%) were found to be positive for HPV 16/HPV 18 infection when PCR based technique was applied. The results indicate, perhaps, a greater specificity of PCR based diagnosis, or presence of other HPV subtypes as etiological factors in the present study group confined to Southern Assam.

Keywords: Fast-PCR - cytological methods - cervical cancer - HPV - sensitivity - specificity - Southern Assam

Asian Pacific J Cancer Prev, **12**, 749-751

Introduction

World-wide epidemiological studies have shown that cancer of the uterine cervix is the second most common malignant disease in women (Ogunmodede et al. 2007). Virtually every cervical cancer (99.7%) is HPV-positive, indicating that the presence of HPV is an obligatory element in the development of cervical cancer (Walboomers et al., 1999). Specific anogenital types of human papillomavirus (HPV) cause the initiating infection that leads to cervical cancer. More than 100 HPV types are known, of which at least 70 infect the anogenital tract. Knowledge of HPV status is becoming increasingly important as a triage screen after detection of atypical cells of undetermined significance (Bollmann et al., 2003) and as a primary screen for cervical cancer detection (Cuzick et al., 2000).

HPV typing has an important prognostic or therapeutic value, as it can distinguish between HPV types of high and low oncogenic risks. Identification of high-risk HPV genotypes may permit selection of those patients who are at increased risk for disease and may therefore provide additional clinical value. An important requirement for this approach is that HPV testing and identification of high-risk HPV types should be highly sensitive and specific (Speich et al., 2004). The types associated with diseases of the anogenital tract can be classified on the basis of phylogenetic relationship (Zur Hausen, 1996) and of associated frequencies with benign or malignant cervical lesions as high-risk types (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -54, -56, -58, -59, and -66) and low risk types (HPV-6, -11, -34, -40, -42, -43, -44). The most common HR types are HPV-16, -18, -31, -33, and -45 (Bosch et al., 1995; Nobbenhuis, 1999). HPV genome can be divided into three different regions including early, late and long control regions (Moosavi et al., 2008). Early region proteins are classified into two groups: E1, E2, E3 and E4 proteins, and E5, E6 and E7 oncoproteins (Dyson, et al., 1989). E5 oncoproteins stimulate the growth of epithelial cells and in many cancers, lead to increased cellular mitosis and consequently cause papilloma lesions (Prayitino, 2006). E6 oncoprotein has been implicated in causing chromosomal abnormalities and progression of cells to neoplasia. E6 is also responsible for causing damage to P53 (Pei, 1996). E7 oncoprotein binds to Rb gene products and other similar proteins thereby inactivating them (Kim, 2001).

The detection and type-specific classification of HPV infection by in vitro viral culture is not possible and serological tests are still ineffective. Direct hybridization based assays, such as Southern blotting and in situ hybridization have been described, but lack sensitivity and specificity (Szuhai, 2001). It has been reported in many studies that target DNA amplification may offer more specificity and sensitivity in detecting HPV infection (Malloy, 2000). In this study we have compared the diagnostic efficacy of PCR based techniques over cytological examination in the direct detection of HPV infection involving HPV 16 and HPV 18 as well as evaluated the relevance as a screening technique.

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Materials and Methods

Samples

Samples were collected from fifty patients attending Cachar Cancer Hospital, Silchar, Silchar Medical College and Hospital, Silchar and different infertility clinics of Southern Assam, who had positive pap smear during May-November, 2010. Considering the short duration of the study, no follow-up was done as part of this study in patients who were found positive for HPV infection by cytological/PCR methods except being referred for further appropriate clinical consultation. Samples were collected with informed consent of subjects.

Cytological Methods

Smears were collected with Ayer's spatula and immediately fixed in absolute alcohol for minimum 6 hours. The smears were then washed with distilled water and dipped in hematoxylene for 7 to 10 minutes. These were subsequently washed in running tap water and dipped in 1% acid alcohol for decolourization, and repeated twice. After third washing, the smears were dipped in 50% alcohol and transferred to O.G 6 solution and kept for 2 to 3 minutes. These were then dipped in absolute alcohol and transferred to EA 50 solution and kept for 20 to 30 minutes. After final dipping in absolute alcohol, the smears were cleared with xylene and mounted for viewing under optical microscope.

DNA extraction

Cervical samples were taken with sterilized swab and put in sterilized collection tubes. The samples were stored at 04°C until DNA isolation. To isolate DNA, sample was taken and mixed with 500μL of TES buffer. Then 30/50μL of 10% SDS and 2/5μL of proteinase K was added to it and kept in a water bath for 1Hr at 57°C. Equal amount of phenol chloroform isoamylalcohol (25:24:1) was added and centrifuged at 12000rpm for 8mins. The supernatant was taken and equal amount of chloroform isoamylalcohol (24:1) was added and further centrifuged. The supernatant was again taken and twice the amount of absolute alcohol was added and kept in refrigerator for some time. The refrigerated supernatant was next centrifuged, alcohol discarded and washed with 200 – 500 μl of 70% alcohol and kept for drying. After that 20 – 50μL nuclease free water was added and left overnight. Isolated DNA was checked under agarose gel electrophoresis in a 1% gel and observed in UV transilluminator for purity.

PCR Method: HPV DNA was demonstrated by performing PCR with consensus primers. To identify the specific HPV types (16 and 18) in the HPV positive specimens further PCR was performed using HPV 16 (5' GCC TGT GTA GGT GTT GAG G 3' -Forward and 5' TGG ATT TAC TGC AAC ATT GG 3' -Reverse) and HPV 18 (5' GTG GAC CAG CAA ATA CAG GA 3' -Forward and 5' TCC AAC ACG TGG TCG TTG CA 3' -Reverse) specific primers. The HPV 16 primer pairs were designed from the L1 region and HPV 18 primer pairs were from the E1 region of the HPV genome. Each amplification reaction mixture of GeneAmp® Fast PCR Master Mix (2X) [Applied Biosystem] 20μL contained buffer, Mg2+, dNTPs, Gold enzyme, sets of Primers and isolated DNA from different cervical samples in 96 wells Veriti Thermal cycler (Applied Biosystem).

FAST PCR amplification using AmpliTaq GOLD (2X):The following PCR steps were employed for FAST PCR (AmpliGOLD-Applied Biosystem) of the total volume of 20 ul.

Step	Reactivation of Enzyme	PCR (30 cycle)			Final step	
	Hold	Denaturation	Annealing	Extension	RAM	Hold
Temp	95°C	95°C	58	68°C	72°C	4°C
Time	10 min	3 sec	3 sec	3 sec	15 sec	15sec

Total Time taken- Reactivation of Enzyme (10 min) + PCR for 30 cycles [3+3+3=9 second X30=270 sec (4½ min)] + 30 sec (½ min)= 15 min

Results and Discussion

In our study, samples were collected from fifty patients as described previously. To avoid false positive results, and more confirmation of HPV genotype, we use this new technique to detect HPV. The mean age of patients was forty five years (ranging from twenty to seventy) and the highest number of HPV positive cases belonged to patients aged forty to fifty years. The presence of amplifiable DNA, using primers for HPV 16 and 18 was confirmed in twenty four (24%) out of the fifty samples. Out of the twelve positive cases HPV type 18 was present in ten cases and HPV type 16 was present in two cases. The PCR was carried out using Fast-PCR method by Applied biosystem reagents (details in Materials and Methods) within in 15 min. with the primers specific to the subtype 16 and 18 and the product sizes were 246 bp (product from L1 region base 379 to 624 with forward – reverse primers) and 162 bp (product from E1 region base 1448 to base 1609 with forward – reverse primers) respectively, depicted in Figure 1.

Our result showed that the occurrence of affected percentage of HPV type 18 is high in comparison to the HPV type 16 in our geographical region. In nineteen cases (76%), it was not possible to identify the virus genotype which we attributed to possibly false positive results of cytological tests or may be due to the presence of other

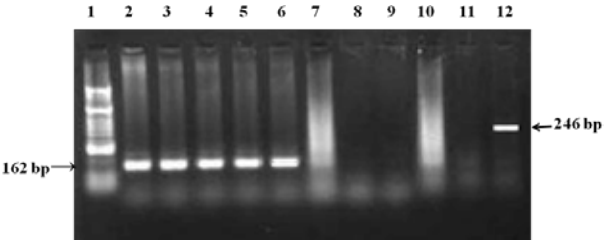

Figure 1. Agarose Gel Electrophoresis (2%) of PCR Products of HPV Type 16 (246 bp) and 18 (162 bp). Lane-1-DNA marker, Lane- 2,3,4,5 and 6-amplified DNA (162 bp) of HPV18 and Lane-7, 8, 9, 10 and 11- no amplified (absence of HPV), 12- amplified DNA (246 bp) HPV type 16

genotypes.

Molecular (PCR) tests may accurately identify different types of HPV (of low and high cancer risks) in cells from cytological screening of cervical lesions and, due to their high sensitivities, have been the focus of attention of many studies (Bauer et al., 1992). The purpose of our study was to detect the HPV 16 and 18 strains by PCR technique with the help of two pairs of primers. The advantage of this PCR based assay, unlike the other currently available assays, is that it is able to report the actual genotype of the HPV detected, rather than issue a broad based ambiguous diagnosis of HPV infection. In the present study, cervical smears were simultaneously subjected to cytological examination as well as PCR technique of detection. The traditional way of classifying tumors is by histopathology. The ability to analyze change in the levels of the transcripts and/or protein products for literally thousands of genes promises interesting possibilities as a research tool for understanding the underlying molecular mechanisms, but also for automated tissue diagnosis (Drain et al., 2002; Oh et al., 2009). HPVs frequently infect humans. They are classified into categories of low risk types responsible for the most common sexually transmitted viral infections and high risk types which are crucial etiological factors in cervical cancer development (McFadden and Schumann, 2001). The direct detection of HPV in cervical specimens may offer an alternative (?) or complement to population-based cytological screening. Recent studies have demonstrated that HPV test results are more sensitive (although they are less specific) than Pap smears in detecting high-grade dysplasia in older women (Schiffman et al., 2000; Wright et al., 2000). In most scenarios women with positive HPV tests still have Pap tests or a diagnostic procedure to provide cytological or histological confirmation of their disease.

We conclude that PCR based diagnosis may be a more specific test for detection of HPV subtypes. Contrary to many studies, it seems that PCR based diagnosis of HPV may not be ideal as a screening test in comparison to conventional pap smear studies, although it may be complementary. However, the use of PCR technique, covering all strains, may be a more sensitive diagnostic procedure that can help to differentiate between suspicious lesions and confirmed HPV infection at risk of subsequent malignancy. We suggest that further studies, covering larger study groups in other geographical locations, are required to draw a firm conclusion as to the diagnostic significance/advantage of PCR techniques over conventional methods.

Acknowledgement

The work was supported by grant from Department of Biotechnology, Govt. of India.

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