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Molecular Analysis of HPV 16 and 18 in Saliva of the Patients with Oral Squamous Cell Carcinoma & Carcinoma of Cervix

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ABSTRACT

The aim of the study was to identify high risk HPV genotypes (HPV 16 and18) among married women in this region. This was a retrospective study conducted from April 2017 to October 2018.A total of 62 cervical samples with high risk HPV, 34 Saliva rinse of OSCC patients from Dept of OBGY Shri B M Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University) Vijayapur, Karnataka, and 180 normal individuals from different parts of Vijayapur, and PCR based high-risk HPV genotyping was carried out. Out of them, 56 cervical cancer patients, 26 OSCC patients and 180 individuals from normal population were positive for HPV infection. Out of 56 HPV positive tissue sample, HPV 16 was observed in 52 (83.87%) samples, HPV 18 was observed in 50 (80.64%) samples. HPV 16 & 18 together was identified in 44 (70.96%) samples. Our results prevails and confirm the infection of HPV high risk genotypes 16 & 18 as major factor in the development of Cervical and Oral squamous cell carcinomas in the population of Vijayapur District.

KEYWORDS: Oral Squamous Cell Carcinoma, Carcinoma of Cervix, HPV

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INTRODUCTION

Cervical cancer is the second most common cancer occurring among women in large areas of the developing countries, where an estimated 80% of new cases arise. Studies in 22 countries, coordinated by the International Agency for Research on Cancer (IARC), and identified HPV DNA in almost all (99.7% of about 1000) cases of cervical cancer[1].

Cervical cancers are caused by different strains of human papilloma virus in which 60% cases of cervical cancer are caused by HPV 16. Previous worldwide data reported, 5, 30,000 women are diagnosed with cervical cancer and nearly 2, 70,000 die from the disease[2]. In India, screening for cervical cancer is carried out cytologically (Pap test). Due to poor health services and high cost involved in the test, it is practically impossible to offer to large female population. The persistent risk of infection is also associated with genotype[3].

Oral Squamous Cell Carcinoma (OSCC) accounts for over 90% of oral cancers[4]. It has reported that smoking, heavy alcohol and betel nut chewing are the etiological factors for OSCC. Advanced research and technology have focused on identifying possible risk factors such as oncogenic human papilloma virus[5].

Till date no reliable or clinically applicable biomarker identified for testing in large population. Therefore, development of saliva based screening test for OSCC may detect at early stage tumors before the development of clinical features. The DNA based direct detection of HPV types may offer an alternative population-based cytological screening[6].

Present study reveals that analysis PCR based high risk HPV genotypes 16 &18 screening in cervical cancer, OSCC in general population using same HPV consensus primers and HPV type 16 & 18 primers.

MATERIALS AND METHODS

Collection of Cervical cancer Samples

This is a retrospective study conducted from April 2017 to October 2018.All the 62 patients included in this study were admitted and being treated at Dept of OBGY Shri B M Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University) Vijayapura, Karnataka, India. Clinically diagnosed patients were screened and confirmed cervical cancer histopathologically, patients were asked to participate in the current study and patients who were comfortable with this study were recruit-

ed, after taking consent in vernacular. A 5mm. tissue by punch biopsy was collected from each patient and was stored in RNA later in -80^{0}C until further use.

Collection of saliva rinse

Clinically and histopathologically confirmed 34 Oral squamous cell carcinoma (OSCC) patient's saliva was collected in sterile 15ml falguen tubes after taking consent of the patients in vernacular from Dept of OBGY Shri B M Patil Medical College, Hospital and Research Centre, BLDE (Deemed to be University) Vijayapura, Karnataka, India. 34 saliva samples from normal individuals were also collected randomly from rural and urban villages & taluks areas, after explaining the purpose of the study. Irrespective of age & sex the samples were collected randomly and high-risk HPV genotyping was carried out by PCR based method at Karnataka Institute of DNA Research (KIDNAR) Karnataka University, Dharwad.

Genomic DNA isolation

Genomic DNA from cervical cancer tissues was isolated by commercially available kit (Bangalore Genei, India) as per manufacturer's instructions. DNA from saliva was isolated by standardized protocol as follows: the samples were centrifuged at 4000rpm for 2min. The supernatant was collected and 250µl of 10% SDS was added and mixed well and 5µl of Proteinase K and RNase H each were added then allowed to stand at room temperature for 45-60 min. To this 150µl of Phenol: Chloroform: Isoamyl alcohol (25:24:1) was added and mixed well, centrifuged at 4000rpm for 5min. Supernatant was collected into 2ml sterile microcentrifuge tubes and chilled absolute alcohol (3 volumes) was added, and centrifuged at 13000rpm for 5 min. DNA pellet was collected, dried and dissolved in 150µl of T50E20 solution. All the DNA samples were confirmed on 0.8% agarose gel and quantified using biophotometer (Eppendorf, Germany).

Polymerase Chain Reaction

HPV consensus, 16 & 18 specific primers were obtained as described in earlier study19. Initially all the samples were screened by HPV consensus primers to select the HPV positive specimens and only the specimens which were positive were tested for HPV 16 & 18 specific types. Genomic DNA isolated from HeLa cell lines were used as positive control in all the PCR reactions. PCR amplification was carried out in a 20µl reaction volume containing 0.5µl of genomic DNA (75ng/µl to 150ng/µl), 0.5µl of each primers (5p.mol.), 0.4µl of dNTP (10p.mol.), 0.2µl of Taq DNA polymerases (3units/µl) along with

Taq buffer 4.0µl(Bangalore GeNei, India) and the total volume was adjusted to 20.0µl using molecular biology grade water. Amplification was carried out in Mastercycler gradient (Eppendorf, Germany) under the following conditions. An initial denaturation at 98ºC for 10sec, followed by 35 cycles at 98ºC for 10sec (cycle denaturation), primer annealing temperature was set depending on the annealing temperature of each primer (14) for 10sec, 72ºC for 15sec (primer extension), and a final extension of 72ºC for 5min. PCR products were confirmed for their respective amplicon size by gel electrophoresis with standard 100bp ladder.

RESULTS

Sixty two patients with cervical cancer, thirty four patients with OSCC and 180 normal individuals were included in this study. Out of them, 56 cervical cancer patients, 26 OSCC patients and 180 individuals from normal population were positive for HPV infection. Table no 1 represents the prevalence and distribution of high-risk HPV types in cervical cancer tissues: Out of 62, 56 HPV positive tissue samples (90.3%), 06 HPV positive tissue sample (9.67%), HPV 16 was observed in 52 (83.87%) samples, HPV 18 was observed in 50 (80.64%) samples. HPV 16 & 18 together was identified in 44 (70.96%) samples. Therefore, in this study we have evaluated the prevalence of high risk HPV types 16 & 18 among cervical cancer patients (n=62), OSCC patients (n=34) and normal individuals (n=180) to determine the risk of cancer development in the population due to HPV infection. Clinically certified cervical cancer tissues, saliva samples of OSCC patients and saliva samples of general population was taken for PCR based analysis of HPV infection. HPV prevalence was observed to be 90.3% (n=56/62) in case of cervical cancer tissues among which prevalence of HPV 16 (83.87%, 52/62) was observed to be higher than HPV 18 (80.64%, 50/62). In case of OSCC patients, 76.47% (n=26/34) HPV prevalence was observed, among which HPV 18 infection (38.23%, 13/34) was observed to be higher than HPV 16 (29.41%, 10/34).

Table 1. Prevalence of HPV Types in Cervical Cancer

	Number	**Total Percentage (%)**
Total Sample Size	62	
HPV Positive	56	90.3
HPV Negative	06	9.67
HPV 16	52	83.8
HPV 18	50	80.6
HPV 16 AND 18	44	70.96

Table 2. Prevalence of HPV Types in OSCC Patients

	Number	Total Percentage (%)
Total Sample Size	34	
HPV Positive	26	76.4
HPV Negative	08	23.5
HPV 16	10	29.4
HPV 18	13	38.2
HPV 16 and 18	02	5.8

Table 3. Prevalence of HPV Types in General Population

	Number	Total Percentage (%)
Total Sample Size	180	
HPV Positive	113	62.7
HPV Negative	67	37.2
HPV 16	08	4.4
HPV 18	76	42.2
HPV 16 and 18	134	74.4

DISCUSSION

Cancers associated with HPV infection frequency is increasing day by day and it is high time to call for a vaccination and eradication or other supportive clinical trials across India. In order to develop such measures we first need to evaluate the prevalence of HPV high risk genotypes in different parts of India. HPV infections and high risk HPV type-specific persistence were found to be high in young married women[7].

Our evaluation showed a slightly higher HPV prevalence in cervical cancer tissues compared with saliva samples of OSCC. Higher cases of multiple infections of HPV 16 & 18 were also seen in case of cervical cancer in comparison with OSCC.

In general population of Bijapur district of Karnataka, HPV 16 and 18 genotype was found to be most frequently distributed compared to HPV 16 and 18 genotype which has higher frequency as observed in the study by A Pavani, et al[8], in Andhra population, Suyamindra S Kulkarni et al[6], North Karnataka population, Kabekkodu et al[9], in coastal Karnataka region.

Another study p53 codon 72, polymorphism and HPV 16 and 18 were seen in OSCC with low frequency in OSF. Frequency of homozygous genotype is at high risk in the presence of HPV 16 and 18 in developing OSCC.[10]

The studies at Turkey and U.K. show lesser prevalence of HPV. The prevalence of HPV 16 is observed to be higher than HPV 18 which is in agreement with the studies conducted globally[11]. In case of OSCC it is observed that in our study the prevalence of both HPV 16 and HPV 18 is quite higher than what was observed in the study conducted by Mahnaz Saheb et al.[12] From these observations it is evident that, there is quite a high percentage of HPV prevalence even in the general population of north Karnataka of Vijayapura in comparison with the studies conducted in India as well as studies of global HPV prevalence. The differential distribution of HPV genotypes in different regions of India could provide a better perspective for the development of appropriate HPV vaccination programme in India.

Chowdhary at al. in 2018 studied twenty cases of OSCC and twenty age matched controls were analyzed to ascertain the prevalence of HPV types 16 and 18. Their results conclude that HPV was higher in cases compared to controls.[13]

CONCLUSION

Our results prevails and confirm the infection of HPV high risk genotypes 16 & 18 as major factor in the development of Cervical and Oral squamous cell carcinomas in the population of north Karnataka as well as Vijayapura District. The high prevalence of HPV genotypes in general population suggests towards vaccination for HPV genotypes as an essential measure for reducing cancer risk due to HPV infection.

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