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Original Article

Detection of Human Papilloma Virus in Saliva of Oral Precancerous and Cancerous Patients

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ABSTRACT
Background: Oral cancer is the fifth common cancer in the world, culminating in over 600,000 new patients annually.Presence of HPV- 16 and 18 in malignant lesions suggests its importance as high risk factor for oral carcinogenesis. The aim of our study was to evaluate the presence of human papillomavirus (HPV) 16/18 in saliva rinses of patient with oral precancerous lesions and oral cancer and to analyse the possibility of using saliva as a diagnostic tool for screening high-risk patients. **Materials & methods:** Group I Pre cancer Group: This group included 20 saliva samples of clinically and histopathologically diagnosed cases of Oral precancerous lesions. Group II Cancer Group: This group included 20 saliva samples of clinically and histopathologically diagnosed cases of Oral cancer. Group III Normal Group: This group included 20 saliva samples of normal healthy patients, age and sex matched controls. Saliva of oral precancerous and cancerous lesion patients sample was collected by 10ml normal saline rinses that was gargled and expectorated. The funnel was removed; the collection tube is sealed with a cap. All the samples were sent to laboratory for further testing. DNA Extraction was using commercial kit from QIAGEN and PCR. **Results:** In the cancer group out of the 20 patients 1 was found to be positive for HPV 16 DNA.(5%). In the precancer group out of the 20 patients1 was found to be positive for HPV 16 DNA.(5%). In the control group out of the 20 patients 0 were found to be positive for HPV 16 DNA. (0%). In the cancer ,precancer and control group all the samples were found to be negative for HPV 18 DNA. The distribution of No. of copies of Viral DNA / Genome / Cell did not differ significantly between two study groups.P-0.667 (Non-Significant). **Conclusion:** In terms of HPV therapeutics, a major focus of the research community could be done in targeting E6 and E7.
Key words: HPV, leukoplakia, oral cancer.

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INTRODUCTION

Oral cancer is the fifth common cancer in the world, culminating in over 600,000 new patients annually. Oral cancer accounts for approximately 30-40% of all cancers in India.[1, 2]

Prevention and early detection of such potentially malignant disorders (PMDs) have the potential of not only decreasing the incidence but also in improving the survival of those who develop oral cancer. Saliva is considered a mirror of body health and is composed of variety of analytes from systemic sources that reach the oral cavity through various pathways. Human papilloma virus (HPV) belongs to a large family of viruses, the papovaviridae, and thus far, more than a 100 different types have been identified in humans. [3, 4]

Presence of HPV- 16 and 18 in malignant lesions suggests its importance as high risk factor for oral carcinogenesis. Application of a real-time PCR technique

in determination of HPV DNA level in head and neck tumor and premalignant tissues as well as in serum has been evaluated. Recent studies investigated HPV DNA in saliva rinses from patients with OSCC, but there is a controversy.[5- 7]

The aim of our study was to evaluate the presence of human papillomavirus (HPV) 16/18 in saliva rinses of patient with oral precancerous lesions and oral cancer and to analyse the possibility of using saliva as a diagnostic tool for screening high-risk patients.

MATERIALS & METHODS

The study entitled Detection of Human papilloma virus 16/18 in saliva of oral precancerous and cancerous patients was conducted in the Department of Oral Medicine and Radiology. The study includes twenty (20) oral cancer patients, twenty (20) patients with oral pre cancerous lesion (leukoplakia) and twenty (20) patients with normal mucosa.

The subjects were grouped as follows:-

Group I Pre cancer Group: This group included 20 saliva samples of clinically and histopathologically diagnosed cases of Oral precancerous lesions

Group II Cancer Group: This group included 20 saliva samples of clinically and histopathologicallydiagnosed cases of Oral cancer

Group III Normal Group: This group included 20 saliva samples of normal healthy patients, age and sex matched controls

Patients were made to sit comfortably on a dental chair. Clinical examination was carried out wearing sterile hand gloves and mouth mask under artificial illumination, with patient seated appropriate to the procedure being performed.Saliva of oral precancerous and cancerous lesion patients sample was collected by 10ml normal saline rinses that was gargled and expectorated. The funnel wasremoved; the collection tube is sealed with a cap. All the samples were sent to laboratory for further testing. DNA Extraction was using commercial kit from QIAGEN and PCR. All the statistical results were done using Chi-Square test. P values were obtained in which only those values were significant which were <0.05.

RESULTS

Distribution of type of HPV 16 and 18 by: Qualitative analysis- in between three study groups

- In the cancer group out of the 20 patients 1 was found to be positive for HPV 16 DNA.(5%).
- In the precancer group out of the 20 patients1 was found to be positive for HPV 16 DNA.(5%).
- In the control group out of the 20 patients 0 were found to be positive for HPV 16 DNA. (0%).
- In the cancer ,precancer and control group all the samples were found to be negative for HPV 18 DNA.
- The distribution of HPV 16/18 did not differ significantly between three study groups.
    - Cancer vsprecancer P-0.999.
    - Cancer vs control P-0.311.
    - Precancervs control P-0.311.
- The number of copies of viral DNA /genome /cell was calculated.
- Cancer group was found to be 2.60 ± 0.70
- In the precancer group values were found to be 1.70 ± 0.85
- The distribution of No. of copies of Viral DNA / Genome / Cell did not differ significantly between two study groups.P-0.667 (Non-Significant)

E6 and E7 changes found in quantivativeanalyeis

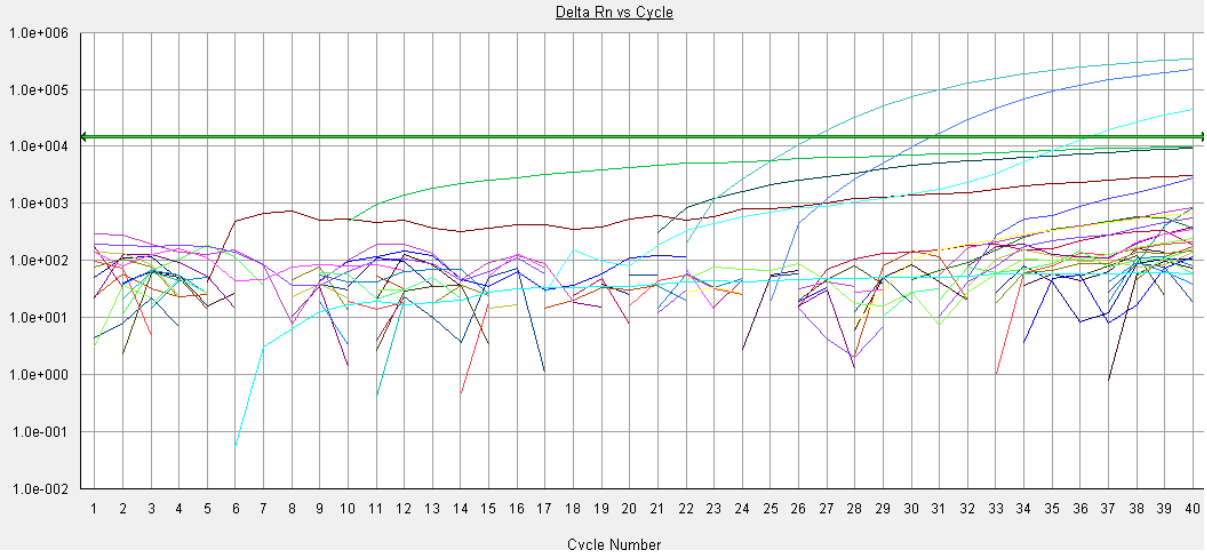
Sample	HPV-16 DNA E6 E7 copies /genome/cell	
PreCancer sample F	E6 3.1	E7 2.1
Cancer sample M	E6 2.3	E7 1.1

The distribution of HPV 16/18 between three study groups.

HPV 16/18	Cancer (n=20)	Pre Cancer (n=20)	Normal (n=20)	P-values		
				Cancer v/s Pre Cancer	Cancer v/s Normal	Pre Cancer v/s Normal
16/18 Negative	19 (95.0)	19 (95.0)	20 (100.0)	0.999 (Non-Significant)	0.311 (Non-Significant)	0.311 (Non-Significant)

The distribution of Quantitative values of HPV 16 DNA between two study groups.

	Cancer Group	Pre Cancer Group	P-value
			Cancer v/s Pre Cancer
No. of copies of Viral DNA / Genome / Cell	2.60 ± 0.70	1.70 ± 0.85	0.667 (Non-Significant)



DISCUSSION

HPV 16

In our study , in 20 control saliva group we found out that all were negative for HPV 16. Our findings matched with the study done by Deidre O Turner et.al. (2010)who reported low prevalence of HPV 16 in saliva.[8] Our findings did not match with MehnazSaheb who reported high prelvalence of HPV 16 in saliva of control group.[9]Our findings also did not match with Suyamindra S Kulkarni (2011) who proposed high prevalence of HPV genotypes in general population of India.[10]

In the 20 cancer group , we found out only 1 out of the 20 patients was positive for HPV 16 which was 5 %. Our Prevalence of HPV 16 in saliva found to be lower as compare with study done by MahnazSahebJamee et .al(2009)[9], who found 27 % HPV16 positivity in saliva of OSCC (n-22). However ,Suyamindra S Kulkarni (2011)[10] proposed high prevalence of HPV 16 in saliva of OSCC that was 70.6% (n-24). In the 20 precancer group, we found only 1 patient was positive for HPV 16.(5%) According to Zhao et al.[11] ,MahnazSahebJamee et .al (2009)[9] who reported that in superficial scrapes and saliva rinses ,HPV DNA is more likely demonstrated in potentially malignant oral lesions.

HPV 18

In control Group, no prevalence OF HPV18 (0%) was seen , Our finding were similar to MahnazSahebJamee et .al (2009)[9] who reported,no prevalence of HPV18 (0%) . In Cancer Group ,all the samples were negative for HPV 18 in saliva , Our findings can be correlated with Juan Du et.al.(2012) MahnazSahebJamee et .al (2009)[9] who found Low prevalence of HPV 18 in oral cancer that is 1% .Our findings contraindicated with Suyamindra S Kulkarni (2011)[10] who observed, high prevalence of HPV 18 in OSCC that is 45.83% (n-11/24) .

In PrecancerGroup ,all the sample were negative for HPV 18 in saliva.

In the cancer group 1 patients who found to be positive for HPV 16 had the habit of smoking and tobacco chewing. Our findings did not match with Ming Zhao[11] who found out higher HPV 16 positive rate for non-smokers (57%) in comparison to smokers (23%) . Our finding did match with Schwartz et al (2001)[12] who proposed that there was potential interaction effect with HPV expression smokers stated and Sinha et al.,(2011) proposed that genetic or epigenetic alterations caused by tobacco have also been postulated to accelerate disease progression in HPV-infected individuals.[13]

In the Precancer group 1 patients who was found to be positive for HPV 16 . Patient had the habit of tobacco chewing. MyongSoo Kim, (1993) hypothesis proposed that oral cancer is induced by sequential exposure of normal oral keratinocytes to "high risk" HPV and tobacco-related carcinogens. MyongSoo Kim, (1993) who Reported that exposure of normal human oral keratinocytes to HPV-16 can generate cells with limited tumorigenicity. The overall low prevalence of HPV 16 and absence of 18 in saliva may not necessarily mean that it does not play a role in progression towards the oral cancer, but more research needs to be done about its role as contributory or risk factor in addition to tobacco. [14, 15]

CONCLUSION

In terms of HPV therapeutics, a major focus of the research community could be done in targeting E6 and E7. Indeed, an agent that could target these oncogenes would be ideal. This would be similar to targeted therapies recently developed for chronic myeloid leukemias (bcrabl targeting) and lung cancer (epidermal growth factor receptor mutations). By these breakthroughs HPV cancers could be treated with different approaches with different modalities such as gene therapy and antiviral therapy

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