Scholars Journal of Applied Medical Sciences (SJAMS)
Abbreviated Key Title: Sch. J. App. Med. Sci.
©Scholars Academic and Scientific Publisher
A Unit of Scholars Academic and Scientific Society, India
www.saspublishers.com

ISSN 2320-6691 (Online)
ISSN 2347-954X (Print)

Microbiology

Comparative Evaluation of Oral Brushing and Oral Rinsing Samples for Detection of Human Papillomavirus in Patients with Oropharyngeal Squamous Cell Carcinoma

Dr. Rahul Agarwal[1], Dr. Jitendra Tiwari[2], Dr Bharti Malhotra[3*]
[1]Resident, Department of Microbiology, SMS medical college and attached group of Hospitals, Jaipur, India
[2]Research Scientist, Department of Microbiology, SMS medical college and attached group of Hospitals, Jaipur, India
[3]Senior Professor, Department of Microbiology, SMS medical college and attached group of Hospitals, Jaipur, India

Original Research Article

***Corresponding author**
Dr Bharti Malhotra

Article History
Received: 21.07.2018
Accepted: 03.08.2018
Published: 30.08.2018

DOI:
10.36347/sjams.2018.v06i08.008

Abstract: Squamous cell carcinomas (SCC) of the oral cavity and oropharynx are amongst the commonest cancers in India. High risk human papillomavirus (HR-HPV) status is a good prognostic marker for patients with oropharyngeal SCC. The present study was undertaken to compare the positivity for HPV in oral brushing versus oral rinsing sample in patients with oropharyngeal SCC. The present study was a laboratory based descriptive observational study. The HR-HPV was detected by Hybrid Caputre 2 (HC2) HPV system as per manufacturer instructions. Statistical analysis was done by using ANOVA. Out of 50 oral brushing and oral rinsing samples, 8 (16%) samples and only 1 (2%) sample were found positive, respectively. The mean age of HC 2 positive patients were found to be not significant as compared to negative cases. Application of Chi square test showed that HC 2 positivity in male (16.7 %) as compared to female (12.5%) were not statistically significant (p=0.817). Maximum HC 2 positivity was seen in lesions located at tonsils (21.4%), followed by lesions located at soft palate (18.2%) and at base of tongue (15%); and none were positive at buccal mucosa. Tobacco chewing, smoking and alcohol consumption were more commonly associated with HC 2 negative patients than HC 2 positive patients. HPV positivity by oral rinsing was quite low in comparison to oral brushings. HC2 may be a useful method for detection of HR-HPV in patients with oropharyngeal SCC and its important to carry out regular HPV testing on such patients as oropharyngeal SCC which are HPV positive respond better to radiotherapy and have better overall survival.
Keywords: HPV, HR-HPV, SCC, oral brushing, oral rinsing.

INTRODUCTION

Squamous cell carcinomas (SCC) of the oral cavity and oropharynx are amongst the commonest cancers in India. Common etiological factors are tobacco chewing, smoking and alcohol consumption. Oropharyngeal carcinomas include carcinoma of the base of tongue, tonsils, and soft palate. However, approximately 20% of oropharyngeal cancers occur in patients lacking these established risk factors and there is strong epidemiologic and experimental evidence indicating that Human papillomavirus (HPV) may account for the majority of these, non-tobacco use cancers [1, 2].

HPV status is a good prognostic marker for patients with oropharyngeal squamous cell carcinoma (OSSC). HPV-positivity is associated with a lower risk of tumor progression and death, due to enhanced sensitivity to ionizing radiation with or without chemotherapy. Studies are presently being done to assess if treatment intensity and duration can be reduced in this subgroup of patients [3].

Commonly, sample is collected from patients by scraping with a specific brush over the lesion. Sometimes, because of the extreme friability of the lesion, the procedure can cause bleeding and pain to the patient. There is a need to find less invasive and non painful methods for sample collection. Therefore, we planned the present study to compare the positivity for HPV in oral brushing versus oral rinsing sample in patients with oro-pharyngeal squamous cell carcinoma.

MATERIALS AND METHODS

Study Area and Site

The laboratory part of study was conducted in SMS Medical College, Jaipur. Samples were obtained from histo-pathologically confirmed case of oro-pharyngeal squamous cell carcinoma patients attending the OPD at SMS hospital, Jaipur.

Study Design

This was a laboratory based descriptive type of observational study.

Sample size

The calculated sample size was 50 cases at 10% allowable error with 95% confidence level.

Inclusion Criteria

Oral brushing as per standard protocol and oral rinsing samples were collected from newly diagnosed, chemotherapy and radiotherapy naïve oropharyngeal SSC patients.

Exclusion Criteria

Patient who had undergone surgery, chemotherapy or radiation for treatment of lesion

Sample collection

Informed consent was taken for taking samples and personal data was collected using a specific form. An oral brushing sample was obtained by brushing the visible lesion with a brush-tipped swab stick. An oral rinsing sample was obtained by gargling the water and spitting into a paper cup. These samples were transferred to STM (Specimen Transport Medium).

Methodology

The specimens were tested for HPV DNA detection according to the standard HC2 method employing the usual 1 pg/mL cutoff. HC2 is a signal-amplified hybridization microplate assay for the chemiluminescent detection of HPV types of low risk (6/11/42/43/44) and high risk (16/ 18/ 31/ 33/ 35/ 39/ 45/ 51/ 52/ 56/ 58/ 59/ 68) as described in the product insert.

Specimens containing the target DNA hybridize with a specific HPV RNA probe. The resultant RNA: DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA: DNA hybrids.

Immobilized hybrids are then reacted with alkaline phosphates conjugated antibodies specific for the RNA: DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted which is measured as relative light units (RLUs) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen. The specimens with an RLU/cutoff value ratio of 1 or greater were considered positive [4].

RESULTS

In our study, out of 50 oral brushing samples, 8 (16%) samples were found positive and out of 50 oral rinsing samples, only 1 (2%) sample was found positive for HR-HPV.

The mean age of HC 2 positive patients was less (47.8 years) as compared to HC 2 negative cases (53.1 years). However, application of unpaired t test showed that this difference was not statistically significant (p=0.243) (Table I).

Out the 8 female subjects, HC 2 was positive in only 1 (12.5%) female, where as HC2 was positive in 7 (16.7%) out of 42 male subjects. Application of Chi square test shows that this difference in HC 2 positivity in relation to sex of subjects was not statistically significant (P=0.817). (Table II).

Maximum HC 2 positivity was seen in lesions located at tonsils (21.4%), followed by lesions located at soft palate (18.2%). Out of the 17 Lesions located at Base of tongue, only 3 (15%) were positive. Five patients had lesion involving Buccal Mucosa and none were HC 2 positive. Application of Chi square test however showed that this difference in HC 2 positive lesion at different sites was not statistically significant (p>0.05). (Table III).

In our study, smoking, tobacco chewing and alcohol consumption was more in HR-HPV negative patients than in HR-HPV positive patients. However these results were not statistically significant (Table IV).

All of the HC 2 negative patients had single sex partner, where as in HC 2 positive cases, 87.5% patients had single sex partner and only 1 patient (12.5%) had multiple sex partners. Application of Chi square test revealed that this difference was not statistically significant (Table IV).

Table-I: Comparison of mean age in HC 2 positive and HC 2 negative cases

Group	N	Mean	Std. Deviation
HC 2 Positive	8	47.8	8.2
HC 2 Negative	42	53.1	12.1

Unpaired t test: t = -1.183 with 48 degrees of freedom; P = 0.243 (NS)

Table-II: HC 2 test results in relation to sex of study subjects

Sex	HC 2 Positive		HC 2 Negative		Grand Total	
	N	%	N	%	N	%
Female	1	12.5	7	87.5	8	100
Male	7	16.7	35	83.3	42	100
Grand Total	8	16	42	84	50	100

Chi-square = 0.054 with 1 degree of freedom; P = 0.817 (NS)

Table III: HC 2 test results in relation to site of lesion

Site of lesion	**HC 2 Negative**		**HC 2 Positive**		**Grand Total**	
	N	%	N	%	N	%
Base of tongue	17	85	3	15	20	100
Soft palate	9	81.8	2	18.2	11	100
Buccal mucosa	5	100	0	0	5	100
Tonsil	11	78.6	3	21.4	14	100
Grand Total	42	100.0	8	100	50	100

Chi-square = 1.313 with 3 degrees of freedom; P = 0.993 (NS)

Table-IV: Correlation of HPV positivity with high risk behavior

Habits	Yes/ No	HC 2 Negative		HC 2 Positive		Grand Total	
		N	%	N	%	N	%
Tobacco chewing	Yes	38	90.5	6	75	44	88
	No	4	9.5	2	25	6	12
Alcohol consumption	Yes	29	69.0	5	62.5	34	68
	No	13	31.0	3	37.5	16	32
Smoking	Yes	37	88.1	7	87.5	44	88
	No	5	11.9	1	12.5	6	12
Multiple sex partner	Yes	0	0	1	12.5	1	2
	No	42	100	7	87.5	49	98

DISCUSSION

Sample collection can be a difficult and time consuming task in patients with oropharyngeal squamous cell carcinoma [5]. In this study, we used oral brushing and oral rinsing samples for detection of HR-HPV in patients with oropharyngeal SCC by hybrid capture 2. This assay may offer some advantages over other detection strategies that it was performed on cytologic specimen which can be procured using minimally invasive techniques. Specimens could be easily collected without the need for tumor micro dissection, formalin-fixation, or specimen processing of any kind. HPV status is a powerful prognostic indicator for patients with oropharyngeal SCC and its important to carry out regular HPV testing on such patients as oropharyngeal SCC which are HPV positive respond better to radiotherapy.

In our study, we compared the oral brushing sample from oral rinsing samples for detection of HR-HPV by hybrid capture 2. In oral rinsing samples, less positivity was likely due to an insufficient number of exfoliated cells being collected in the rinse fluid (as opposed to directly sampling the gross lesion by brushing), producing a specimen too dilute to reach the threshold of HPV detection by HC2 [6]. Thus, HC2 analysis of oral rinsing specimens did not seem to be useful for detecting HPV-related oropharyngeal SCCs.

CONCLUSION

HPV positivity was found to be significant in our patients with opharyngeal SCC. As HPV-related oropharyngeal carcinoma tend to be more sensitive to radiation and chemotherapy, this investigation should be conducted in all patients and there is the need for routine HPV testing of all oropharyngeal carcinoma patients. As of date, the standard sampling method remains brush cytology. For the oral rinse method, which is less traumatic and much more patient friendly, to become clinically relevant we need to significantly enhance the sensitivity of the test so as to pick low level virus DNA in the diluted oral rinse sample.

REFERENCES

1. Klussmann JP, Gültekin E, Weissenborn SJ, Wieland U, Dries V, Dienes HP, Eckel HE, Pfister HJ, Fuchs PG. Expression of p16 protein identifies a distinct entity of tonsillar carcinomas associated with human papillomavirus. The American journal of pathology. 2003 Mar 1;162(3):747-53.
2. Venuti A, Badaracco G, Rizzo C, Mafera B, Rahimi S, Vigili M. Presence of HPV in head and neck tumours: high prevalence in tonsillar

localization. Journal of experimental & clinical cancer research: CR. 2004 Dec;23(4):561-6.

3. Tahtali A, Hey C, Geissler C, Filman N, Diensthuber M, Leinung M, Stoever T, Wagenblast J. HPV status and overall survival of patients with oropharyngeal squamous cell carcinoma–a retrospective study of a German head and neck cancer center. Anticancer research. 2013 Aug 1;33(8):3481-5.
4. Kulmala SM, Syrjänen S, Shabalova I, Petrovichev N, Kozachenko V, Podistov J, Ivanchenko O, Zakharenko S, Nerovjna R, Kljukina L, Branovskaja M. Human papillomavirus testing with the hybrid capture 2 assay and PCR as screening tools. Journal of clinical microbiology. 2004 Jun 1;42(6):2470-5.
5. Messadi DV. Diagnostic aids for detection of oral precancerous conditions. International journal of oral science. 2013 Jun;5(2):59.
6. Jarboe EA, Willis M, Bentz B, Buchmann L, Hunt J, Ellis G, Layfield L. Detection of human papillomavirus using hybrid capture 2 in oral brushings from patients with oropharyngeal squamous cell carcinoma. American journal of clinical pathology. 2011 May 1;135(5):766-9.