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Prevalence of HPV/p16+ infection among oropharyngeal squamous cell carcinoma patients in a tertiary care centre in Southern India

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Introduction. Oropharyngeal squamous cell carcinomas are often found to be associated with human papilloma virus (HPV) infection. The prevalence of HPV infection among oropharyngeal squamous cell carcinomas patients in India is comparatively lower to that of the same worldwide.

Aim. To find out the prevalence of HPV infection among oropharyngeal squamous cell carcinomas patients who presented in our hospital.

Settings and design. Retrospective cross-sectional study.

Materials and methods. Tissue block of 60 patients with biopsy-proven oropharyngeal squamous cell carcinomas were subjected to immunohistochemistry for evaluating p16 expression. The p16 expression pattern was correlated with the demographic details. Data was entered in Microsoft Excel and Statistical Analysis was done with the help of SPSS version 22 (IBM Corp. Released, 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.).

Results. Prevalence of HPV infection in our study was found to be 11.7 %. 85.8 % of all p16-positive patients had moderate-well differentiated disease. 6 out of 7 p16-positive patients had higher T stage (T3–4). All the patients who were p16+ were found to have a higher Nodal stage (N2–3). 100 % of all p16+ patients were found to have stage IV disease.

Conclusion. Prevalence of HPV infection was found to be similar to that of previous studies conducted in India. These patients also presented with advanced nodal disease at presentation and thereby, an advanced overall stage.

Keywords: human papillomavirus infection, oropharyngeal cancers, prevalence of infection, Indian subpopulation

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Распространенность инфекции HPV/p16+ среди пациентов с орофарингеальной плоскоклеточной карциномой в центре высокоспециализированной медицинской помощи в Южной Индии

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Введение. Орофарингеальная плоскоклеточная карцинома часто связана с заражением вирусом папилломы человека (ВПЧ). Распространенность инфекции ВПЧ среди пациентов с данной патологией в Индии сравнительно ниже, чем в других странах.

Цель исследования – определить распространенность инфекции ВПЧ среди пациентов с орофарингеальной плоскоклеточной карциномой, получавших лечение в нашей больнице.

Дизайн исследования. Ретроспективное одномоментное исследование.

Материалы и методы. Образцы тканей 60 пациентов с подтвержденным биопсией диагнозом «орофарингеальная плоскоклеточная карцинома» были исследованы иммуногистохимически на экспрессию p16. Было проведено сравнение профиля экспрессии p16 с демографическими данными. Полученные результаты оценены с помощью программы Microsoft Excel. Статистический анализ выполнен с использованием программного обеспечения SPSS version 22 (IBM Corp. Released, 2013. IBM SPSS Statistics для Windows, версия 22.0, Армонк, Нью-Йорк: IBM Corp.).

Результаты. Частота встречаемости ВПЧ в нашем исследовании составила 11,7 %. Средне- и высокодифференцированное заболевание наблюдалось у 85,8 % пациентов с положительной экспрессией p16. Шесть из 7 больных с такой экспрессией p16 имели более высокую T-стадию (T3–4), а все больные с таким показателем – высокую N-стадию (N2–3). У всех пациентов с положительной экспрессией p16 наблюдалось заболевание стадии IV.

Заключение. Распространенность инфекции ВПЧ была близкой к значениям, полученным в других исследованиях в Индии. Для пациентов с этой инфекцией также были характерны вовлеченность лимфатических узлов (N-стадия) в патологический процесс и более высокая общая стадия заболевания.

Ключевые слова: инфицирование вирусом папилломы человека, орофарингеальный рак, распространенность инфекции, индийская субпопуляция

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INTRODUCTION

Head and neck cancers approximately accounts for 900,000 cases worldwide with over 400,000 deaths annually. They comprise 30 % of all malignancies in India [1]. Consumption of tobacco products and alcohol are the typically associated risk factors [2].

Oropharyngeal squamous cell carcinomas (OPSCC) are often found in association with human papilloma virus (HPV) infection. They generally present with advanced nodal disease at presentation. However, they respond better to definitive treatment [3].

Increased HPV viral load noted in OPSCC leads to increased expression of oncoproteins E6 and E7, causing suppression of p53 and pRb, which indirectly causes overexpression of p16. This increased expression of p16 is known to be an excellent surrogate marker of active transforming infection of high-risk HPV types [4]. Hence, immunohistochemical staining of p16 is considered a highly sensitive detection method for active HPV infection [5].

This study was conducted to find out the prevalence of HPV infection in OPSCC patients by evaluating the p16 expression by immunohistochemistry (IHC) in our centre.

MATERIALS AND METHODS

Study design. A total of 60 oropharyngeal histopathological specimens of OPSCC patients who presented in our centre from 2017 to 2022 were available for final evaluation and were selected based on the following inclusion/exclusion criterion.

Inclusion criteria: patients who presented with the diagnosis of OPSCC in our centre.

Exclusion criteria:

- all head and neck carcinomas except OPSCC;
- incomplete medical records;
- inadequacy/unavailability of histopathological specimens.

Ethical clearance: patient consent was taken, and the institutional ethical committee approval was obtained “IEC KMC MLR 12–2020/419”.

Patients were staged according to the 7th edition of American Joint Committee on Cancer (AJCC) Tumor, Nodus and Metastasis (TNM) classification of malignant tumours, using their respective clinical notes and radiological imaging records, as the p16 expression assessment was done afterwards. Tumour and lymph node status were divided into two categories (T1–2 and T3–4; N0–1 and N2–3, respectively). Other demographic data were also noted.

Staining procedure. All the biopsy slides were initially reviewed, and appropriate blocks with the maximum tumour content were chosen for immunohistochemistry. 3-micron thin sections were cut from the tumour blocks. These sections were incubated for 1 hour at 80 degrees Celsius. The antigen retrieval solution was prepared and pre-heated in the microwave oven for 5 minutes. The slides were dipped in this pre-heated solution, incubated for 20 minutes, and then allowed to cool. Then the slides were washed under running tap water, after which they were transferred to distilled water. After this step, a blocking agent (a buffered casein solution with sodium azide) was added to the solution and incubated for 10 minutes. The slides were again washed with distilled water and dipped in a wash buffer solution, which was made separately, for 10 minutes. After this, the primary antibody for p16 (clone MX007, “Dako”) was added to the slides and incubated for 40 minutes at room temperature. After incubation, slides were washed in the wash buffer for 10 minutes. Then the linker solution was added to the slides and incubated for 20 minutes at room temperature. Following this, the slides were again washed with wash buffer for 10 minutes. After this step, the secondary antibody, horse radish peroxidase (HRP), was added to the slides and incubated for 30 minutes at room temperature. After 30 minutes, the slides were washed in the wash buffer for 10 minutes and then treated with a freshly prepared diaminobenzidine (DAB) solution, following which the slides were incubated for 5 minutes at room temperature. Next, the slides were washed with a wash buffer for 5 minutes. Finally, counterstaining was performed with Meyer’s haematoxylin for 3 minutes.

Positive control was used for the verification of the marker, the staining procedure, and the expression pattern on the respective slides. The strong, diffuse, and homogenous pattern of expression of p16 in both cytoplasm and nuclei was considered positive p16 expression.

Statistical analysis. Data was entered in Microsoft Excel and Statistical Analysis was done with the help of SPSS version 22 (IBM Corp. Released, 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). A descriptive analysis was used to indicate the distribution of the quantitative variables in terms of mean, standard deviation (SD)/median, inter-quartile range (IQR). The qualitative variables were defined in terms of percentages by category. Association between p16 expression and demographic data was calculated using the Chi square test. p -value of <0.05 was considered as significant value.

RESULTS

Demographic data. A total of 60 patients were included for final evaluation in this longitudinal study. The median age in this study was found to be 60 years (mean \pm SD = 58.27 ± 10.09) (table 1). Majority of the patients were found to be males with a male: female ratio of 9: 1 (table 2). The commonest subsite among the parts of the oropharynx involved in this study was found to be the tonsil (48.3 %) followed by the base of tongue (36.7 %) (table 2). Histopathological examination revealed that majority of the patients (60 %) had moderately differentiated squamous cell carcinoma (MDSCC) followed by well-differentiated squamous cell carcinoma (WDSCC) (30 %) and poorly differentiated squamous cell carcinoma (PDSCC) (10 %) (table 2).

In this study, 50 patients (83.4 %) had advanced T stage (T3–4) and 47 (78.4 %) had advanced N stage (N2–3) (table 2). 46 out of 60 patients (76.7 %) had stage IV disease. 18.3 % had stage III disease, 55 had stage II disease.

Table 1. Demographic data (median 60, mean \pm SD 58.27 ± 10.09 ; range 47)

Parameter	Number of patients	
	<i>n</i>	%
Age, years:		
<60	29	48.3
>60	31	51.7
total	60	100.0

Prevalence and correlations. Out of the 60 total patients in the study, 7 patients (11.7 %) were found to be p16-positive (HPV-associated) (table 3, figure). 85.8 % of all p16-positive patients had moderate-well differentiated disease whereas 90.6 % of all p16-negative patients had moderate-well differentiated disease. 6 out of 7 p16-positive patients had higher T stage (T3–4). 83.1 % of p16-negative patients had T3–4 stage. All the patients who were p16+ were found to have a higher nodal stage of N2–3. Whereas only 75.5 % of the p16– patients were found to have N2–3 nodal stage. 100 % of all p16+ patients were found to have

stage IV disease. 73.6 % of p16– patients were found to have stage IV disease (table 4).

Table 2. Clinical data

Parameter	Number of patients	
	<i>n</i>	%
Gender:		
male	54	90
female	6	10
Subsite:		
base of tongue	22	36.7
soft palate	8	13.3
tonsil	29	48.3
uvula	1	1.7
Histological grade:		
WDSCC	18	30
MDSCC	36	60
PDSCC	6	10
T stage:		
T1–2	10	16.6
T3–4	50	83.4
N stage:		
N0–1	13	21.6
N2–3	47	78.4
Overall stage:		
I	0	0
II	3	5
III	11	18.3
IV	46	76.7

Note. Here and in table 4: WDSCC – well-differentiated squamous cell carcinoma; MDSCC – moderately differentiated squamous cell carcinoma; PDSCC – poorly differentiated squamous cell carcinoma.

Table 3. Prevalence of human papilloma virus

p16 status	Number of patients	
	<i>n</i>	%
Negative	53	88.3
Positive	7	11.7
Total	60	100.0

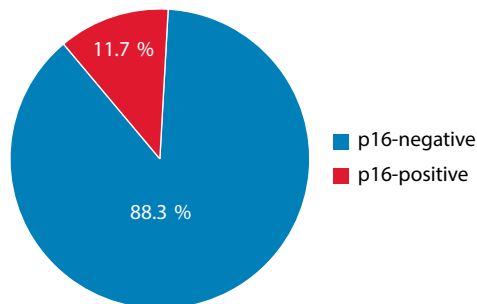
DISCUSSION

It is clear from previous studies that there is a significant association between HPV infection and head and neck cancer, especially in OPSCC. The prevalence of this HPV infection is generally seen higher in the western population when compared to that of Indian population.

B.J. Cline et al. conducted a study including 31702 OPSCC patients in which the mean age at diagnosis was found to be 60.3 years. In this study, 22.9 % were females and 77.1 % were males [6]. Similarly in our study, the mean

Table 4. Correlations with human papilloma virus status

Parameter	p16-positive (%)	p16-negative (%)	p-value
Histological grade:			
WDSCC	42.9	28.4	0.616
MDSCC	42.9	62.2	
PDSCC	14.2	9.4	
T stage:			
T1–2	14.2	16.9	0.857
T3–4	83.1	85.8	
N stage:			
N0–1	0	24.5	0.139
N2–3	100	75.5	
Stage:			
I	0	0	0.299
II	0	5.6	
III	0	20.8	
IV	100	73.6	

*Prevalence of human papilloma virus*

age at diagnosis was found to be 58.2. Out of the total 60 patients, 54 of them (90 %) were males, and the remaining 6 patients (10 %) were females. A study done by Tristantham et al which included 23297 OPSCC patients had tonsil as the most common subsite (96.4 %) and poorly differentiated squamous cell carcinomas as the most common histology (38.9 %) [7]. Our study also had tonsil as the most common subsite (48.3 %) followed by the base of tongue (36.7 %). Majority of the patients had moderately differentiated squamous cell carcinomas as the most common histological grade in our study (60 %) followed by well-differentiated squamous cell carcinomas (30 %).

According to the Centers for Disease Control and Prevention (CDC), among the US population, 70 % of the OPSCC patients are associated with HPV infection. C. de Martel

et al. conducted a study in 2008 including 12.7 million cases to quantify the burden of cancers associated with infections worldwide. The pattern of HPV prevalence among oropharyngeal carcinoma (OPC) patients from various regions in this research was as follows: 56 % in North America, 39 % in Northern & Western Europe, 38 % in Eastern Europe, 17 % in Southern Europe, 45 % in Australia, 52 % in Japan, and 13 % in the remaining countries combined [8]. A study by A. Bahl et al. in India revealed 22.8 % of patients had HPV-associated disease among the total 105 patients with OPC [2]. Studies conducted by V. Murthy et al. and M.K. Sannigrahi et al. including OPC patients from Indian subpopulation also had similar rates of prevalence – 20 % and 15 % respectively [9, 10]. The prevalence of HPV in our study was found to be 11.7 %. The slight variations in prevalence rates amidst the various studies conducted may be attributable to the technique of detection chosen, which may be HPV DNA *in-situ* hybridization (ISH), HPV DNA polymerase chain reaction (PCR), or IHC for the interpretation of p16 expression.

In a study by N. Wakisaka et al., 35.8 % patients had advanced T stage (T3–4), 60.3 % had higher N stage (N1–3) and 75.4 % had advanced stage disease (stage III–IV). Among the HPV-positive patients in this study, 31.8 % had T3–4 stage, 77.2 % had N1–3 disease and 81.8 % had stage III–IV disease [11]. Out of the total 60 patients in our study, 83.3 % had T3–4 stage, 78.3 % had N2–3 stage and 76.6 % had stage IV disease. In the HPV-positive patients in our study, 85.8 % had T3–4 stage, 100 % of the patients had N2–3 disease and 95 % had stage IV disease.

In summary, our study demonstrated an HPV-positivity rate of 11.7 % among the 60 total patients taken up for evaluation. Similar to previous literature, the HPV-positive patients in our study presented with advanced nodal disease at presentation and thereby, an advanced overall stage. Further follow-up of these patients including their treatment details, determination of possible prognostic markers, and evaluation of their survival parameters can be done which can help in modifying the existing treatment modalities as HPV-associated OPSCC are known to have better prognosis according to literature.

CONCLUSION

Prevalence of HPV infection was found to be similar to that of previous studies conducted in India. These patients also presented with advanced nodal disease at presentation and thereby, an advanced overall stage.

R E F E R E N C E S / Л И Т Е Р А Т У Р А

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Authors' contribution

Manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work, if that information is not provided in another form.

Вклад авторов

Текст статьи был прочитан и одобрен всеми авторами, указанные выше в данном документе требования авторства были удовлетворены, и все авторы считают, что статья отражает реальную работу, если эта информация не представлена в другой форме.

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All patients gave written informed consent to participate in the study.

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