

Original Research Article

A study of HPV association in upper aerodigestive tract cancer by immunohistochemical analysis of p16

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Abstract: Context: The high risk HPV 16 and 18 are known to be associated with some cancers of UADT, particularly oropharynx. These are more commonly seen in young patients, predominantly in male without known risk factors like smoking and alcoholism. Clinically they present with small or occult primary tumor with advanced neck disease. These patients are sensitive to radiotherapy and have better prognosis than conventional keratinizing SCC. The p16 is a surrogate marker for detection HPV.

Aims: To detect the frequency of p16 positivity in UADT cancer and correlate p16 positivity with the clinical presentation, histological type and grade of UADT cancers.

Methods and Material: Observational study conducted from January 2016 to June 2017 on all the newly diagnosed cases of primary UADT cancer confirmed by histopathological examination. Tissue biopsies were processed, 5 micron sections were stained with H and E and 3 micron sections were subjected for p16 IHC. The collected data was entered in Excel sheet and analyzed using Microsoft Excel software for percentages and proportions.

Results: The UADT cancer study subjects showed 37.2% positivity for p16. They were in the age group of 41 to 50 years, most of them were male. Majority of p16 positive UADT cancer study subjects were non smokers, non alcoholics and were not chewing betel nuts. Common site of involvement is oropharynx. **Conclusions:** Analysis of our result with review of literature showed significant comparable p16 positivity with oropharyngeal cancers. The p16 immunohistochemistry is a surrogate marker for detection of HPV.

Keywords: Upper aerodigestive tract cancers; HPV; P16; IHC; Oropharynx.

1. Introduction

The major known risk factors for the upper aero digestive tract (UADT) cancer are tobacco and alcohol [1]. Approximately 20% of head and neck cancers occur in people lacking these established risk factors [2]. Human papilloma virus (HPV) appears to be an independent risk factor in a subset of UADT cancers [1].

HPV positive UADT cancers are distinct clinically and pathologically from smoking and alcohol related classic SCC [3]. Frequently seen in young patients less than 40 years with a male to female ratio of 4:1 [4]. They usually present as a small or occult primary tumor with advanced neck disease [3].

The route of transmission of HPV in UADT cancers is still poorly understood. Promiscuity does not seem to be an important risk factor for UADT cancers. A clear correlation between the HPV type in oral cavity and in anogenital tract in women and their male partner is not been established [5].

HPV associated squamous cell carcinoma (SCC) can occur anywhere in upper aerodigestive tract. Frequently seen in oropharynx especially at the base of tongue and palatine tonsil. These tumors are also been noted in the sinonasal tract, oral cavity, nasopharynx, hypopharynx, and larynx [6].

These patients are sensitive to radiotherapy and have better prognosis than conventional keratinizing SCC. The p16 is a surrogate marker for detection HPV [6].

The study was conducted to detect p16 positivity in upper aerodigestive tract cancers by immunohistochemistry and to correlate the p16 positivity with clinical presentation, histological type and grade of UADT cancer.

2. Materials and Methods

It is an observational study conducted in the central laboratory of Department of Pathology at Mandya Institute of Medical Sciences, Mandya from January 2016 to June 2017 (for a period of 18 months).

2.1. Inclusion criteria

- All age groups, both male and female.
- All the newly diagnosed cases of primary UADT cancer confirmed by histopathological examination.

2.2. Exclusion criteria

- Patients with known UADT carcinomas treated with radiation and chemotherapy.
- Cancers of salivary gland.

Clinical data for each patient was obtained after taking informed written consent from patients. After adequate fixation of the specimen, tissue bits were subjected for processing and paraffin embedding. Three-to-four-micron thick sections were deparaphinized and stained with haematoxylin and eosin (H & E). The histological typing and grading was done.

2.3. Histologic Grade

- GX: Cannot be assessed
- G1: Well differentiated
- G2: Moderately differentiated
- G3: Poorly differentiated

Table 1. Percentage score

Percentage of stained tumor cells	Scores
<5%	1+
5% to 20%	2+
21% to 50%	3+
51% to 100%	4+

Table 2. Intensity score

Intensity	Scores
Equal to no staining	1
Low intensity	2
Moderate	3
High intensity	4

The 3 micron thickness tissues sections were used for immunohistochemical staining to determine the p16 expression. The p16 staining is both nuclear and cytoplasmic marker. The p16 expression was scored based on the percentage and intensity of nuclear and cytoplasm staining (Table 1 and 2). The percentage score of 4+ for tumor cells (51% to 100%), with intensity score of 3 or 4 is considered positive for p16 over expression [7].

3. Results

Data obtained was tabulated and analyzed. Out of 43 study subjects of UADT cancer, 16 (37.2%) of them showed positive staining for p16 and 27(62.8%) showed negative staining for p16 (Table 3).

Table 3. Distribution of p16 expression of the study subjects

p16 expression	No of cases	Percentage
p16 Positive	16	37.2
p16 Negative	27	63.8
Total	43	100

Out of 16 p16 positive UADT cancer study subjects 14 (87.5%) were men and 02 (12.5%) were women. In p16 negative UADT cancers all 27 (100%) study subjects were men.

The age of study subjects with p16 positive upper aero digestive tract cancers ranged from 34 to 85 years. Majority of cases 07(43.7%) were in the age group of 41 to 50 years and 02(12.5%) of cases were seen in <40 years of age. p16 negative upper aero digestive tract cancers ranged from 30 to 85 years. Majority of cases 10 (37.0%) were in the age group of 51 to 60 years.

Out of 16 p16 positive upper aero digestive tract cancers, 02 (12.5%) were smokers, 03 (18.7%) were alcoholics, 03 (18.7%) patients had betel nut chewing habit and none of them had history of oral sex or multiple partners. Out of 27 study subjects with p16 negative upper aero digestive tract cancers 27 (100%) were smokers, 25(92.5%) were alcoholics, 24 (88.8%) patients had betel nut chewing habit.

Majority of the patients with p16 positive upper aerodigestive tract cancers presented with mass with throat pain 10 (62.5%), followed by neck swelling 05(31.2%), dysphagia 04 (25.0%), hoarseness of voice 02 (12.5%), trismus 01 (6.2%), proptosis and diplopia 01 (6.2%).

Majority of study subjects with p16 positive UADT cancer presented with cancer of oropharynx 10 (62.5%), followed by supra glottis 03 (18.7%), nasopharynx 01 (6.2%), oral cavity 01 (6.2%) and hypopharynx 01 (6.2%). Study subjects with p16 negative UADT cancer presented with cancer of supra glottis 09 (33.3%), followed by oropharynx 06 (22.2%), hypopharynx 06 (22.2%), oral cavity 03(11.1%), glottis 02 (7.4%) and esophagus 01(3.7%) (Table 4).

Table 4. Site of involvement of cancer in study subjects according to p16 expression

Anatomical site	p16 Positive (%) n=16	p16 negative (%) n=27
Oropharynx	10 (62.7)	06 (22.2)
Supra glottis	03 (18.7)	09 (33.3)
Nasopharynx	01(6.2)	00
Oral cavity	01(6.2)	03(11.1)
Hypopharynx	01(6.2)	06 (22.2)
Glottis	00	02 (07.5)
Oesophagus	00	01(03.7)
Total	16(100)	27(100)

In the present study all cases of UADT cancer both p16 positive and p16 negative were keratinizing squamous cell carcinoma. Majority of the patients with p16 positive UADT cancers were Grade II carcinomas 14 (87.5%), followed by Grade I carcinomas 02 (12.5%). The p16 negative UADT cancers presented with 24 (88.8%) Grade II carcinomas, followed by 02 (7.4%) grade I carcinomas and 01(3.7%) Grade III carcinomas.

4. Discussion

Several studies have investigated the HPV association in UADT cancer.

In the present study, 16 (37.2%) out of 43 cases were positive for p16 comparable with other studies, by Josen K. Stephen and co-workers [8], Rebecca D and co-workers [9], Elaine M. Smith and co-workers [10] with p16 positivity of 26.5% (21 of 80 cases), 32.2% (8 of 28 cases), 38% (89 of 237 cases) respectively. Ralli and co-workers [11], Haitham Mirghani and co-workers [12] showed higher p16 positivity of 78.6% (59 out of 75 cases) and 68% (34 out of 50 cases) respectively (Table 5).

Table 5. Showing p16 expression of UADT cancer study subjects in our study in comparison with other studies

Study	p16 positive	p16 negative
Megha Ralli <i>et al.</i> ,(2016) [11]	78.6%	21.4%
Josena K. Stephen <i>et al.</i> ,(2013)[8]	26.5%	73.5%
Haitham Mirghani(2015)[12]	68%	32%
Rebecca D <i>et al.</i> ,(2010)[9]	32.2%	67.8%
Elaine M Smith <i>et al</i> (2010)[10]	38%	62%
Present study	37.2%	62.8%

In the present study, majority of UADT cancer subjects were men (87.5%), rest were women (12.5%) similar to study conducted by Elaine M. Smith and co workers [10] with 61% of men and 39% of women, Megha Ralli and co-workers [11] with 85.3% of men and 14.7% of women, Josen K. Stephen and co-workers [8] study having 76.1% of men and 23.9% of women, Rebecca D and co-workers [9] with 64.5% of men and 35.5% of women.

In the present study, the age group ranged from 35- 84 years with the mean age of 59.5 years, majority of them were between 41 to 50 years and 12.5% them were below 40years, which was similar to studies by Megha Ralli *et al.*, [11] (range 27 to 75 years with mean of 54.7 ± 11.3 years), Rebecca D *et al.*, [9] (range from 40 to 68 years with mean of 54 years), Elaine M Smith *et. Al* [10] (mean age 60 years), Zhongchuan Will Chen [11] (range 43 to 92 years with mean of 61).

In the present study 12.5% of study subjects had habit of smoking, 18.7% of them were alcoholics, 18.7% of them were chewing beetle nut and no significant association of oral sex or multiple partner was observed. Extracting such history from the patients in our society was not very easy as it is considered a social taboo. In the study conducted by Megha Ralli *et al.*, [11] 86% of the study subjects were smokers, 61% of them were alcoholics, 28% of them had habit of chewing beetle nut, oral sex was seen in 10.6% and 22.6% had multiple partners. Similarly 71.4% of study subjects of Josena K. Stephen *et al.*, [8] were smokers.

The common site of involvement in our study is oropharynx 62.5%, similar to study conducted by Megha Ralli *et al.*, [11] with 80%, Josena K. Stephen *et al.*, [8] with 61.9% and Elaine M Smith *et al.*, [10] with 68%. In our study p16 positive oropharyngeal carcinoma was followed by supra glottis with 18.7%, nasopharynx with 6.2%, oral cavity with 6.2%, hypopharynx with 6.2%. In the study conducted by Megha Ralli *et al.*, [11] oropharyngeal carcinoma(80%) was followed by glottis carcinoma with 10.6% and oral cavity with 9.3%, Josena K. Stephen *et al.*, [8] with 61.9% oropharyngeal carcinoma followed by 19.2% oral cancer and Elaine M Smith *et al.*, [10] with 68% of oropharyngeal cancers followed by 28% of oral cancers.

In our study majority 87.5% of p16 positive study subjects showed grade II squamous cell carcinoma followed by grade I that is 12.5% similar to study conducted by Megha Ralli *et al.*, [11] with 83.4% of grade II cancers.

5. Conclusion

The HPV associated upper aerodigestive tract cancers are the cause of significant morbidity and mortality in middle age men. They significantly affect the vital functions like breathing, swallowing and speech. The high risk HPV types like 16 and 18 are most commonly associated with UADT cancers.

The p16 immunohistochemistry is a surrogate marker for detection of HPV. The p16 positive UADT cancers are known to have good prognosis. Upper aerodigestive tract is the site of second primary tumor in patients with HPV associated cancer anywhere.

Our study evaluated p16 positive immunoreactivity and correlated with clinico pathological parameters like age, sex, clinical presentation, site, habits, histological type and grade. A significant association was found between p16 positivity and oropharyngeal cancers. HPV association is frequently seen in oropharyngeal carcinoma. Although no significant association of p16 positivity with age, histological type and grade was noted. This perhaps is attributed to the study of limited number of cases.

In conclusion, the p16 IHC is a rapid and cost effective technique for detection of HPV that can be easily adopted in all Pathology laboratories.

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Conflicts of Interest: "The authors declare that they do not have any competing interests."

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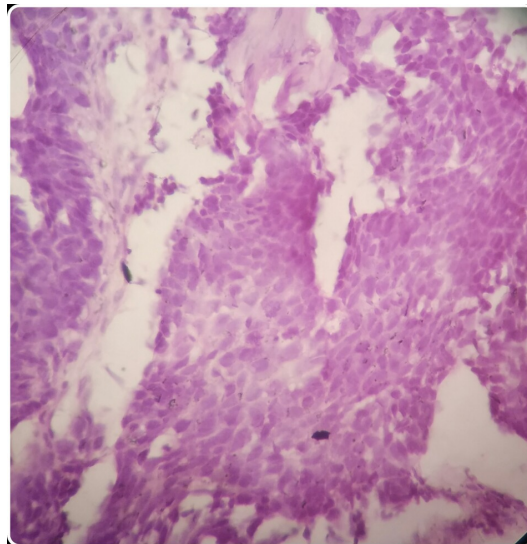


Figure 1. Moderately Differentiated SCC of Oropharynx (H and E stain, 40 X)

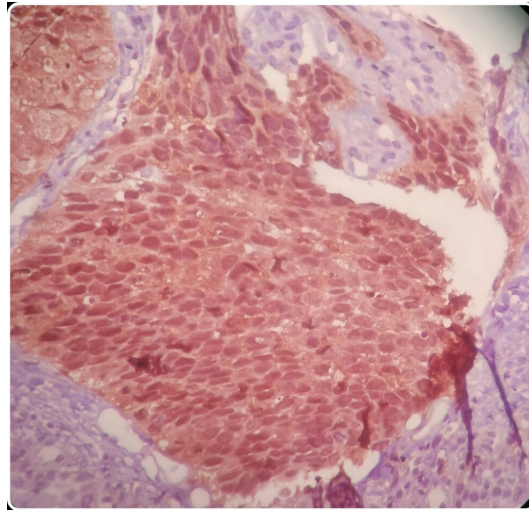


Figure 2. p16 positive Moderately Differentiated SCC of Oropharynx (IHC, 40X)

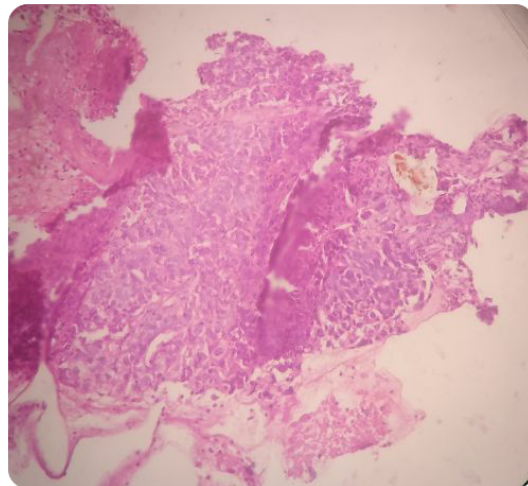


Figure 3. Moderately Differentiated SCC of Nasopharynx (H and E stain, 10 X)

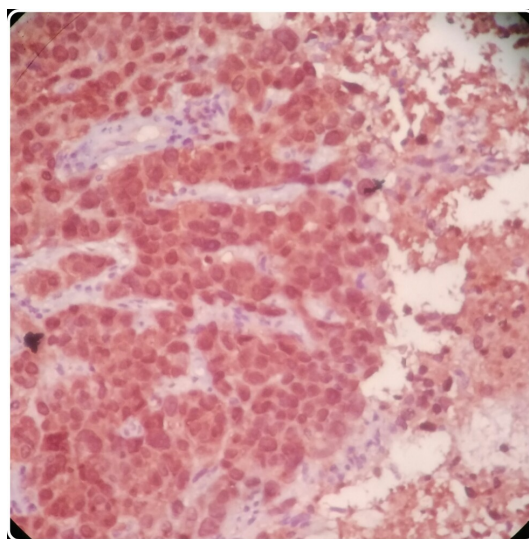


Figure 4. p16 positive Moderately Differentiated SCC of Nasopharynx (IHC, 40X)

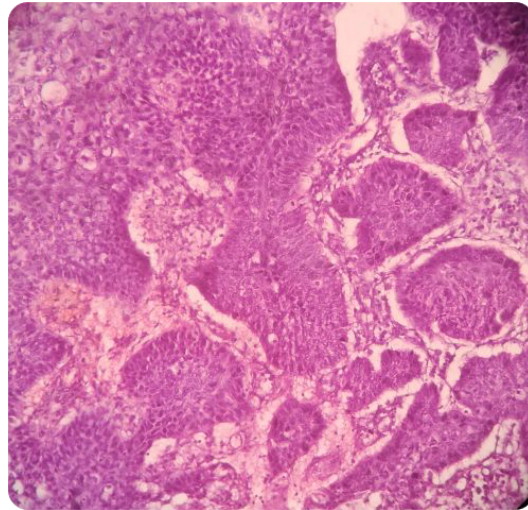


Figure 5. Well Differentiated SCC of glottis (H and E stain, 40 x)

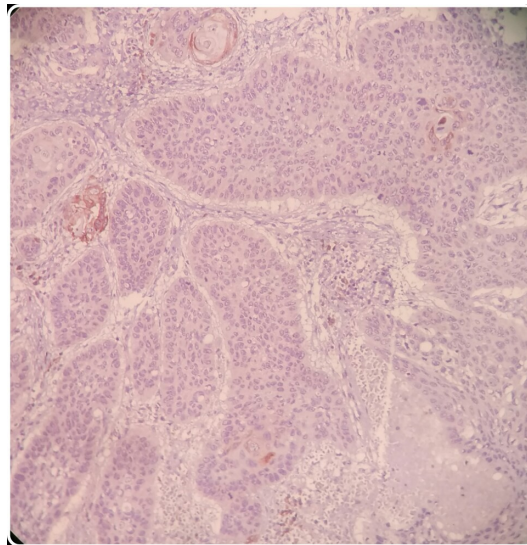


Figure 6. p16 negative Well Differentiated SCC of Glottis (IHC, 10X)



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