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Recent Trends in p16-Positive Oropharyngeal Squamous Cell Carcinoma in Northern Israel

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Abstract

Background

The proportion of oropharyngeal squamous cell carcinoma (OPSCC) that is p16-positive has increased worldwide, yet the proportion in Israel is unknown. We aimed to evaluate trends and outcomes of p16-positive OPSCC, as assessed by p16 immunohistochemistry (IHC), in northern Israel.

Methods

This retrospective study included patients diagnosed with OPSCC who were treated at a single tertiary center during 2012–2024. Collected data included demographics, p16 IHC status, staging, treatment modalities, and outcomes. Tumor staging was performed according to the American Joint Committee on Cancer 8th Edition.

Results

The study included 130 patients (108 males) with known p16 IHC status. The mean age was 61.6 ± 10.5 years, and mean follow-up was 33.9 ± 26.9 months. Seventy-two percent (n = 94) of patients were p16-positive, with the proportion of p16-positive cases increasing temporally from 61% (2012–2016) to 92% (2023–2024). Clinically, the p16-positive and p16-negative cohorts were of similar age; however, p16-positive tumors were more frequently localized to the tonsil and presented at significantly lower T and N stages. Treatment modalities varied by cohort, with induction chemotherapy and concurrent cetuximab administered more frequently to p16-negative patients. Notably, 5-year overall survival was significantly higher in the p16-positive cohort (75% vs. 47%, $p < 0.001$).

Conclusions

Over the past decade, p16-positive OPSCC averaged 72% in Israel, yet averaged 78% for the last four years. This mirrors trends in North America and Northern Europe. Although preliminary, during 2023–2024, 92% of OPSCC diagnoses were p16-positive.

Background

The global incidence of oropharyngeal squamous cell carcinoma (OPSCC) has increased markedly in recent decades and currently has the fastest growing incidence among head and neck cancers in high-income countries [1]. While the historical risk factors for head and neck cancers have been tobacco and alcohol consumption, human papillomavirus (HPV) has emerged as a major etiological agent, driving a marked shift in the epidemiology of OPSCC [2–4]. Substantial geographic variation has been described, in both overall OPSCC incidence and in the proportion of HPV-positive cases [5]. In the UK and USA, the

incidence of HPV-related OPSCC in men now surpasses that of cervical cancer in women, which is primarily caused by high-risk HPV (HR-HPV) [6].

HPV-related compared to HPV-negative OPSCC typically affects younger, non-smoking individuals, often of higher socioeconomic status, and is more prevalent in high income countries [7, 8]. p16 immunohistochemistry (IHC) acts as a surrogate marker of HPV infection; also identified as p16INK4a, cyclin-dependent kinase inhibitor 2A (CDKN2A). Patients with p16-positive OPSCC typically respond better to radiotherapy and chemoradiotherapy, with substantially higher overall survival and progression-free survival rates [9–12].

To date, only one study has evaluated the epidemiology of OPSCC and the proportion of HPV-positive disease in Israel. There, among 74 patients diagnosed with OPSCC during 1999–2011, HPV DNA was detected in 33.7%. Using p16 IHC, the rate was 36%. The proportion of HPV-related OPSCC was about threefold lower in Israel than in other Western countries [13].

The present study aimed to assess the evolving epidemiology of p16-positive OPSCC in northern Israel. We examined temporal changes during 12 years, in p-16 status, as assessed by p16 IHC; and assessed the factors associated with the outcomes, among patients treated at a single tertiary referral center that serves as the sole provider of comprehensive head and neck oncology services for northern Israel.

Patients and Methods

Study design and population

This retrospective cohort study included adult patients (≥ 18 years) with histologically confirmed OPSCC who were treated between June 2012 and May 2024 in Rambam Health Care Campus, the sole comprehensive head and neck oncology center for northern Israel.

The northern region of Israel comprises two districts: the Northern and Haifa districts. In 2012, this combined region had a population of 2,282,200 (28.6% of the country's total population). By 2024, the population had increased to 2,698,200 (26.9% of the total population) [14–15].

The patients were treated with intensity-modulated radiotherapy (IMRT), and concurrent chemotherapy was administered when clinically indicated. During the early years of the study, induction chemotherapy, and concurrent treatment with cetuximab were administered to patients with high-stage disease.

The inclusion criteria were OPSCC of all subsites, and oncological treatment. Data were collected from electronic medical records of Rambam Health Care Campus (Prometheus), including patient demographics, tumor characteristics, p16 IHC status, staging, treatment details, and clinical outcomes. The independent variables included demographic factors (age, gender), smoking status, tumor subsite, T and N stage (American Joint Committee on Cancer (AJCC) 8th Edition) [16], p16 IHC status, and treatment modality. The dependent variables were the proportion of p16-positive OPSCC of all the

patients with OPSCC, and outcome. The proportion of p16-positive OPSCC was calculated for each year of the study. Furthermore, due to the small number of patients during the first 5 years of the study period, and to better illustrate the trends, consecutive years were combined as follows: 2012–2016, 2017–2020, and 2021–2024. To evaluate trends between these three periods, *p* for trend was calculated using the linear-by-linear association.

Patients who were initially staged using the AJCC 7th Edition were reclassified for the purpose of this study, according to the updated criteria. Patients with pathologies other than SCC in the oropharynx were excluded.

Immunohistochemistry (IHC) Staining for p16

The College of American Pathologists (CAP) recommends utilizing p16 IHC as a surrogate marker for high-risk HPV (HR-HPV) testing [17]. This assay is widely accessible for routine clinical use and methodologically straightforward. However, because p16 serves primarily as a surrogate biomarker, its upregulation does not universally guarantee the presence of an active HR-HPV infection [17]. Despite this biological limitation, previous studies have demonstrated a strong correlation between p16-positive IHC and molecular HPV DNA detection, with reported correlation coefficients of 0.88 [13]. This study was conducted as a retrospective chart review. Within our cohort, p16 IHC served as the most widely available parameter for HPV evaluation. Direct viral detection via HPV DNA or RNA testing was not routinely performed during the study period and was therefore unavailable for inclusion.

IHC of p16 was done using the fully automated VENTANA BenchMark system (Ventana Medical Systems, Tucson, AZ, USA), p16INK4a immunohistochemical staining was performed on 4-µm, formalin-fixed, paraffin-embedded tumor tissue sections. The pre-diluted CINtec® p16 histology assay (mouse monoclonal antibody clone E6H4; Ventana, Cat. No. 705–4793) was applied according to the manufacturer's protocol. Antigen retrieval was performed using Ventana CC1 buffer (pH 8.4). Detection utilized the OptiView DAB IHC Detection Kit, and slides were counterstained with hematoxylin.

P16 positivity was defined as strong, diffuse nuclear and cytoplasmic staining in ≥ 70% of tumor cells, in line with the College of American Pathologists and AJCC recommendations [17]. Overexpression of p16 is widely used as an established surrogate marker for transcriptionally active high-risk HPV [17].

In the current study, p16 IHC was used to stratify patients with OPSCC to p16-positive vs. p16-negative, and to evaluate associated clinicopathologic characteristics.

Outcomes

The primary outcome was the proportion of patients with p16-positive OPSCC throughout the study period, and associations with clinic-pathologic characteristics. Secondary outcomes included overall survival, defined as the time from diagnosis to death from any cause, or the last follow-up.

Statistical Analysis

Descriptive statistics were used to describe the baseline characteristics of the patients. Numbers and proportions were used to summarize categorical variables; and means, standard deviations, to summarize continuous variables. Demographic and clinical baseline characteristics were compared between patients who were p16-positive and p16-negative, using the Chi Square or Fisher exact test for the categorical variables and the independent *t*-test for age, a continuous variable. Age, the only continuous variable, was evaluated for normality, using the Kolmogorov Smirnov test.

Application of the AJCC 8th Edition criteria resulted in significant downstaging of patients with p16-positive OPSCC. Because disease stage demonstrated a significant statistical interaction with p16 status across all analyses, a composite variable incorporating both p16 status and T classification was utilized. Consequently,the entire cohort was stratified into four categories: 1. p16-positive and early T stage (T1-T2); 2. p16-positive and advanced T stage (T3-T4); 3. p16-negative and early T stage (T1-T2); and 4. p16-negative and advanced T stage (T3-T4).

Univariate Cox regression was used to evaluate associations between baseline characteristics and overall survival. Time to mortality was estimated using Kaplan-Meier survival curves. The association between p16-status combined with T stage, and overall survival was assessed using multivariate Cox regression. The analysis was adjusted for variables with $p < 0.1$ in the univariate analysis. These included age, subsite, radiotherapy protocol including concomitant treatment with either cisplatin, carboplatin or cetuximab, and the combination variable of T stage and p16 status. This method was used to select variables for the multivariate analysis because the relatively small number of events precluded. Missing values were included in all analysis using separate categories. Hazard ratios (HR) with 95% confidence intervals (CI) are shown. The analyses were performed using JMP software and IBM SPSS statistics version 28 (IBM, New York, NY). All the tests were two-tailed. A *p*-value of < 0.05 was considered statistically significant.

Results

Clinical and Demographic data of the study population

A total of 160 patients were diagnosed with OPSCC and were identified from the electronic medical records of Rambam Health Care Campus (Prometheus). Thirty patients were excluded from further analysis. Specifically, 27 patients lacked complete p16 IHC data, and three patients were lost to follow-up. Consequently, the final analytical cohort consisted of 130 patients. Of them, 108 (83%) were males and 22 (17%) females. The mean age at diagnosis was 61.6 ± 10.5 years (median 62, range, 35–93 years). The mean follow-up duration was 33.9 ± 26.9 months (median 26.5 months, range, 3–119 months). In seven patients, the tumor in the oropharynx was a second primary tumor in a previously irradiated neck. Of the 130 patients, 109 (83%) were Jewish and 21 (17%) Arab (Table **1**). The tonsil was the most common subsite (65, 50%), followed by the base of the tongue (45,35%), the soft palate (5, 4%),

and the posterior pharyngeal wall (5, 4%). Fifty-five patients (42%**)** were current smokers, 26 (20%) were former smokers, and 43 (33%) never smokers.

T and N stages according to the AJCC 8th Edition were as follows: 73 (56%) presented with early T stage (T1, T2), and 52 (40%) with advanced T stage (T3–T4). Nodal staging at diagnosis revealed no regional lymph node involvement in 19 patients 15%, N1 disease in 60 (46%), and advanced nodal stage (N2–N3) in 45 (35%). Missing data occurred randomly across the cohort and are summarized in Table 1.

Table 1 Clinical, pathological, and treatment characteristics of the patients according to p16 positivity. The data are presented as number (%) unless stated otherwise.

Parameter	p16-positive – 94 Patients No. (%)	p16-negative- 36 Patients No. (%)	*p*-value
Age (years, mean ± SD)	61.5 ± 11.1	63.1 ± 8.2	0.435
Gender, male	77 (82)	31 (86)	0.568
Ethnicity			
Jews	82 (87)	27 (75)	0.090
Arabs	12 (13)	9 (25)	
Subsite			
Tonsil	53(57)	12 (33)	0.002
Base of the tongue	31 (33)	14 (40)	
Other (soft palate, post wall)	3 (3)	7 (19)	
Missing	7 (7)	3 (8)	
T stage			
T1-2	62 (66)	11 (31)	<0.001
T3-4	29 (31)	23 (64)	
Missing	3 (3)	2 (5)	
N stage			
N0-1	64 (68)	15 (42)	0.005
N2-3	26 (28)	19 (53)	
Missing	4 (4)	2 (5)	
Treatment			
Induction chemotherapy:			
Yes	10 (11)	12 (33)	<0.001
No	80 (85)	19 (53)	
Missing	4 (4)	5 (14)	
Radiotherapy			
Radiotherapy alone (including DHANCA[a] Protocol)	11 (12)	3 (8)	0.037
Radiotherapy concurrent with:			
Cisplatin		18 (50)	
Carboplatin			

Cetuximab		3 (8)	
Missing		7 (20)	
	71 (76) 5 (5) 6 (6)	5 (14)	
	1 (1)		
Smoking			
Current smoker	34 (36)	21 (59)	0.057
Past smoker	19 (20)	7 (19)	
Non smoker	36 (39)	7 (19)	
Missing	5 (5)	1 (3)	

[a]Abbreviation: DHANCA; Danish Head and Neck Cancer group radiotherapy protocol.

Of 130 patients included in the study, 94 (72%) were p16-positive (Table **2**) and 36 (28%) were p16-negative. All seven patients with OPSCC as a second primary tumor in the head and neck, were p16-negative.

Table 2 The proportions of patients who were p16-positive among all the patients with OPSCC, according to periods of diagnosis. Data for patients without p16 IHC results, stratified by year of diagnosis, are also presented in the table.

Period (years)	Total number of patients with p16 IHC	Number of patients p16-positive	Percentage of p16- positivity	Excluded Patients (lack of p16 IHC)
2012-2016	18	11	61	17
2017-2020	52	36	69	9
2021-2024	60	47	78	1
Overall	130	94	72	27

P16 staining among patients

To account for low patient volumes in the early years of the study, data were aggregated into three consecutive time intervals for analysis: 2012–2016, 2017–2020, and 2021–2024. Over the study period, the proportion of p16-positive cases increased from 61% (11/18) in the 2012–2016 cohort to 78% (47/60) in the 2021–2024 cohort, demonstrating a trend of borderline statistical significance (*p*-trend = 0.057).

In the most recent two years (2023–2024), 92% of all the OPSCC diagnoses were p16-positive (22 of 24). These temporal changes are summarized in Table **2**. Table **2** also outlines the cohort of patients excluded from the study due to unknown p16 IHC status. This missing data disproportionately reflects the earlier years of the cohort (2012–2016), which accounted for 19 of the 27 excluded cases, aligning with historical shifts in routine clinical testing practices

Table **1** summarizes the cohort's clinical, pathological, and treatment characteristics according to p16 positivity. No statistically significant differences were observed between the p16-positive and p16-negative groups, in their mean ages and gender distribution. Arabs had a higher proportion of p16-negative OPSCC 9 (43%) vs 27 (25%) Jews (p=0.09), although not significant.

The distribution of primary tumor sites was found to differ according to p16 positivity. In the p16-positive group, the tonsil was the most frequent primary site (53, 57%). In contrast, in the p16- negative group, the tonsil was involved in 33% (12) of the tumors (p=0.002). Among p16- negative cohort 40% (14) of the patients had tumors originating from the base of the tongue.

P16 positivity was associated with disease stage at diagnosis. Among the patients who were p16-positive, 66% (62) had early-stage tumors (T1–T2); while among the patients who were p16- negative, 64% (23) presented with advanced T stage (T3–T4) (p<0.001). A similar trend was observed for nodal involvement: 68% (64) of the patients who were p16-positive had N0–N1 disease, whereas 53% (19) of the patients who were p16- negative presented with advanced nodal disease (N2–N3) (p=0.005). Applying the AJCC 8th Edition guidelines yielded lower T and N stages for patients who were p16-positive.

Oncological treatment

All of the patients received primary oncological treatment. Concurrent chemoradiotherapy was the preferred protocol among both p16-negative (78% of patients) and p16-positive (87% of patients). Radiotherapy as an only treatment was administered to 12% (11) of patients in the p16-positive group, compared with 8% (3) in the p16-negative group. Among patients who underwent combined treatment, the predominant regimen consisted of radiation therapy with concurrent cisplatin. A statistically significant difference was observed in the specific regimens administered in combination with radiation therapy. A lower proportion of p16-positive than p16-negative patients received cetuximab: 6% (6) vs. 20% (7) (p=0.037) (Table **1**). Induction therapy was administered far less frequently in the p16-positive than the p16-negative group 11% (10) vs. 33% (12), p<0.001) (Table **1**).

Furthermore, treatment with induction chemotherapy correlated with treatment with concomitant cetuximab. Six out of 22 (27%) who were treated with induction chemotherapy, received cetuximab as concomitant therapy as compared to 6 out of 87 patients (7%) without induction (p=0.023).

Overall survival

Among the 130 patients in the cohort, 100 (77%) were alive and 30 (23%) had died at the end of the follow-up period. Overall, 94 patients (72%) had no evidence of disease at their last follow-up visits and 6 (5%) were alive with disease. Twenty-six patients (20%) had died of disease, and 4 patients (3%) had died from causes unrelated to their cancer. Overall, five-year survival for the entire cohort was 67%. Five-year survival was better for patients who were p16-positive than p16-negative: 75% vs. 47% (log-rank test, $p<0.001$). The p16-positive cohort experienced 14 events over a median follow-up of 35 months (for surviving patients), whereas the p16-negative cohort experienced 16 events over a median follow-up of 34.7 months.

Seven patients developed a second primary OPSCC; all these cases were p16-negative. Upon excluding this cohort from the survival analysis, the 5-year overall survival rate for the p16-negative group was 54%, compared to 75% for the p16-positive group (log-rank test $p=0.01$).

In univariate analysis (Table **3**), the following parameters were associated with overall survival: tonsil subsite of the tumor ($p=0.03$), lower T stage ($p=0.016$), p16-positive ($p<0.001$), concomitant treatment with cisplatin rather than only radiotherapy ($p=0.007$). P16-negativity with advanced T stage (T3-T4) was associated with higher mortality than p16-positivity with early T stage (T1-T2) (HR 6.1(CI 2.2-16.5) $p<0.001$) (fig 1). [Figure 1 here]. The distribution of the cohort according to the four categories that combine T stage and p16 status, as described above, is presented in Table **3**. The complete-case analysis yielded consistent results compared with the model that included a separate missing data category.

Table 3 Univariate model for overall survival

	Death		HR (95% CI)	*p*-value
	No (N=100)	**Yes (N=30)**		
Age (mean ±SD)	60.7±10.1	63.9±11.4	1.03 (0.99-1.07)	0.096
Gender				
male	84 (84)	24 (80)	reference	
female	16 (16)	6 (20)	1.32 (0.54-3.25)	0.544
Ethnicity				
Jews	83(83)	25 (83)	reference	
Arabs	17 (17)	5 (17)	1.4 (0.53-3.7)	0.498
Subsites				
Tonsil	53 (53)	12 (40)	reference	
Base of the tongue	34 (34)	11 (37)	1.4 (0.62-3.2)	0.408
Others	6 (6)	4 (13)	3.5 (1.1-11)	0.030
Missing	7(7)	3(10)		
T Stage				
T1-2	64 (64)	9 (30)	reference	
T3-4	35 (35)	17 (57)	3.2 (1.4-7.09)	0.006
Missing	1 (1.0)	4 (13)	8.3 (2.5-26.9)	<0.001
Combined p16 and T stage				
p16(+) T1-T2	56 (56)	6 (20)	reference	
p16(+) T3-T4	23 (23)	6 (20)	2.5 (0.79-7.7)	0.122
p16(-) T1-T2	8 (8)	3 (10)	2.8 (0.7-11.3)	0.147
p16(-) T3-T4	12 (12)	11 (36)	6.1 (2.3-16.6)	<0.001
Missing T stage for p16(+)	1 (1)	2 (7)		
Missing T stage for p16(-)	0	2 (7)		
N Stage				
N0-1	61 (61)	17 (68)	reference	
N2-3	37 (37)	8 (32)	0.86 (0.37-1.99)	0.717
Missing (overall 6)	2 (2)	4 (13)		

p16-positivity				
negative	20 (20)	16 (53)	reference	
positive	80 (80)	14 (47)	0.30 (0.15-0.61)	<0.001
Induction Chemotherapy				
no	80 (84)	19 (73)	reference	
yes	15 (16)	7 (27)	1.76 (0.74-4.2)	0.202
Missing (9)	5 (5)	4(13)		
Radiotherapy Protocol				
RT/DHANCA	7 (7)	6 (20)	Reference	
RT Concomitant with:				
Cisplatin	77 (77)	12 (40)	0.26 (0.1-0.68)	0.007
Carboplatin	5 (5)	3 (10)	0.77 (0.19-3.09)	0.709
Cetuximab	6 (6)	7 (23)	0.86 (0.28-2.61)	0.787
Missing	5(5)	2 (7)		
Smoking				
No	34 (34)	9 (30)	reference	
Past	19 (19)	7 (23)	1.5 (0.56-4.1)	0.422
Current	42 (42)	13 (43)	1.2 (0.52-2.8)	0.663
Missing	5(5)	1(3)		

Abbreviations: HR- hazard ratio; CI- confidence interval; RT- radiotherapy; DHANCA- Danish Head and Neck Cancer group radiotherapy protocol; P16(+)- p16-positive; P16(-)- p16-negative.
The data are presented as number (%) unless stated otherwise.

Multivariate model for overall survival

Overall survival differed according to the categories of P16 combined with T stage. In multivariate analysis, the category of p16-negative with advanced T stage (HR 8.1, CI 2.4-27.5, p<0.001) were significant predictors of poorer overall survival while concurrent treatment with Cisplatin was positive predictor for overall survival compared to only radiotherapy (HR 0.13, 0.03-0.48 p=0.002). However, due to a limited number of events in the multivariate model, the resulting hazard ratios may be unstable. (Table **4**).

Table 4 Multivariate model for overall survival

	Events	Median follow-up (months) for surviving patients	Median follow-up (months) for all patients	HR (95%CI)	*p*-value
Combined p16 status and T stage					
P16 (+) Early T stage	6	37.6	30.7	reference	
P16 (+) Advanced T stage	6	42.8	31.2	4.0 (1.13-14.4)	0.032
P16(-) Early T stage	3	36.9	36.9	2.9 (0.72-11.9)	0.133
P16(-) Advanced T stage	11	45.8	16.8	8.1 (2.4-27.5)	<0.001
Radiotherapy Protocol					
RT/DHANCA	6	41.5	28.2	reference	
RT Concurrent with Cisplatin	12	38.5	31.2	0.13 (0.03-0.48)	0.002
RT Concurrent with Carboplatin	3	26.6	23.7	0.23 (0.04-1.26)	0.090
RT Concurrent with Cetuximab	7	100.6	39.2	0.28 (0.07-1.16)	0.078

Abbreviations: HR, hazard ratio; CI, confidence interval; RT- radiotherapy; DHANCA- Danish Head and Neck Cancer group radiotherapy protocol; P16(+)- p16-positive; P16(-)- p16-negative.

Analysis of excluded cases

Twenty-seven patients who lacked p16 staining were excluded from the analysis. Seventeen of them (63%) were from the early years of study (2013-2016), nine (33%) from 2017-2018, and one (4%) from 2020. Their mean age (65.6 ± 8.1 years) was higher than either p16-positive and p16-negative patients, although not significant. Five-year survival rate of patients without p16 staining (50%) was not different from the p16-negative group (p=0.624), and significantly lower than the p16-positive group (p=0.049).

Discussion

The current study shows a trend of a substantially higher proportion of p16-positive than p16- negative OPSCC in northern Israel in comparison to data reported for the whole country one decade earlier [13].

The average proportion of p16-positive OPSCC was 72% during the entire study period, 2012–2024. During the last two years, 2023–2024, the average proportion of p16-positive OPSCC in northern Israel was 91-92% which is higher compared with 34% proportion reported for 2011 for the whole country [13]. However, the current study evaluated p16 expression via immunohistochemistry within our population, whereas Amit et al. assessed HPV DNA status in a cohort of 74 patients. Although there is strong diagnostic concordance between these two methods for high-risk (HR) HPV detection, p16 serves primarily as a surrogate biomarker, and its overexpression does not uniformly guarantee the presence of HR-HPV.

The present findings are consistent with increases in the proportions of p16-positive OPSCC that have been reported in high income countries [18,19]. For example, in the United States, the incidence of HPV-positive OPSCC increased by 225% between 1988 and 2004, and already then, HPV-positive tumors accounted for the majority of OPSCC diagnoses [18]. Similarly, in Denmark, the age-adjusted incidence rate of HPV positive/p16 positive OPSCC rose from 0.9 to 3.5 per 100,000 between 2000 and 2020 [19]. Examination of data from the MD Anderson Cancer Center cohort showed that 88% of OPSCC diagnoses were HPV-positive [20]. Our findings contrast with the lower HPV-attributable OPSCC rates in many Asian countries, which are also reportedly increasing [21,22]. In Hong Kong, Chen et al. reported a rise in HPV-positive tonsillar carcinoma from 40% (2010–2015) to 65% (2016–2020) [21]. Similarly, the BROADEN study in China demonstrated an increase from 44.2% to 51.7% over the decade 2009–2019 [22]. As of 2021, about 33% of OPSCC cases worldwide were HPV-positive, with considerable regional variation ranging from 0% in India, to 68% in the USA, and 70% in Sweden [23]. Taken together, the findings illustrate the global shift toward HPV-driven oropharyngeal cancers, while underscoring that absolute prevalence remains geographically dependent. While most of the aforementioned studies utilized HPV DNA/RNA testing to identify HPV-associated OPSCC, several incorporated p16 IHC. Notably, Carlander et al. [20] and Dona et al [24]. employed both diagnostic modalities, demonstrating strong concordance between HPV molecular detection and p16 expression

The current study observation of a trend of an increasing proportion of OPSCC that are p16-positive parallels the increase of cervical cancer incidence in Israel, from an age-standardized rate of 4.1 to 6.2 per 100,000 between 1985 and 2022 [25]. The latter falls between the 2022 rates in the USA and Denmark: 4.9 and 8.9 per 100,000, respectively. However, despite the increasing proportion of p16-positive OPSCC in Israel, the age-standardized rate for oropharyngeal carcinoma in Israel was very low in 2022, 0.21 per 100,000 (all genders), compared with 2.6 in the USA and 4.1 in Denmark [26]. The proportions of p16-positive OPSCC were shown to differ significantly between Jews and Arabs; the latter may represent a community with a higher proportion of smokers [27].

Taken together, the findings emphasize two key epidemiologic considerations. First, the proportion of p16-positive OPSCC is influenced by geographic, demographic, and behavioral factors within the population. Second, in retrospective analyses spanning multiple years, temporal shifts should be

accounted for, as pooling earlier and more recent data may obscure changing and current incidences of p16-positive diseases. The present study findings align with other reports of temporal increases, such as Dona et al.'s documentation of a rise in HPV-positive OPSCC in Italy, from 40.3% (2010–2014) to 53.7% (2015–2019) [24].

From a clinical standpoint, the current results mirror patterns described in Western cohorts. While no age difference was observed between patients who were p16-positive and p16-negative, significant differences emerged in smoking history, tumor stage, and primary subsite distribution, consistent with established epidemiologic distinctions. The present study showed lower N stage, on average, of patients who were p16-positive compared to p16-negative. However, re-staging all our patients according to the AJCC 8th Edition guideline yielded lower T and N stages for patients who were p16- positive compared to p16-negative patients and compared to the AJCC 7th Edition. The finding of better prognosis, including markedly greater five-year overall survival, among patients who were p16-positive compared to p16-negative, aligns with the literature [28].

It is well established that the prognosis of p16-negative compared to p16-positive OPSCC is considerably worse, and the presentation of multiple comorbidities often precludes the use of cisplatin-based concurrent chemoradiation. Consequently, many such patients receive induction chemotherapy—often aimed at symptom control—followed by radiotherapy only or in combination with cetuximab. This treatment paradigm was evident in the present study cohort, in which a substantial proportion of p16-negative patients underwent induction therapy, and others received concurrent radiotherapy with cetuximab instead of standard cisplatin-based chemoradiation. Furthermore, the p16-negative group included seven patients (19%) who had second primary OPSCC, in a previously irradiated neck. These patients had worse outcomes, and this contributed to the more adverse outcomes of the p16-negative group. However, given the retrospective nature of this study, treatment allocation was not randomized. Consequently, the improved outcomes observed in patients treated with cisplatin rather than cetuximab may reflect selection bias rather than an independent treatment effect.

Designating a combined parameter of p16 status and T stage demonstrated that p16-negative and advanced T stage was the most significant predictor for poorer overall survival, in addition to treatment with concurrent cetuximab, which also represents more advanced disease.

The apparent negative impact of concurrent cetuximab observed in our cohort is consistent with high-level evidence from randomized controlled trials. In both De-ESCALaTE HPV and NRG Oncology RTOG 1016, radiotherapy plus cetuximab resulted in poorer overall survival or worse disease control compared with radiotherapy plus cisplatin [29,30]. Notably, survival outcomes in our cohort appeared inferior even when compared with the cetuximab arm of the NRG Oncology RTOG 1016. This finding is biologically and clinically plausible given the predominance of p16-positive OPSCC in the current cohort, alongside the presence of a subset of p16-negative e oropharyngeal carcinomas, which are associated with more aggressive disease biology and poorer prognosis. Other potential contributors to these differences include variations in radiotherapy delivery—specifically the accelerated regimen of 70 Gy delivered over a

6 week-period in the NRG Oncology RTOG 1016—as well as the retrospective, non-randomized nature of the present analysis.

Strengths and limitations of the study

The current study is the largest single-center study on OPSCC and the first to evaluate temporal changes of p16-positive proportions during the last decade in Israel. In the past, the rate of HPV-related cancer was low in Israel, and the current study may indicate changes in lifestyle that were not evaluated herein. The study also shows differences in the pattern of p16-positive OPSCC in Israel, among patients older than those described in North America and Europe.

This study's main limitations are related to the biases inherent in the retrospective design. As so, clinical data regarding subsite, T and N stage and treatment are missing in small percents of patients and are transparently documented in the tables.

The sample size was small, and the data were from one cancer center in Israel, which mainly treated patients from the northern part of the country. Furthermore, 27 patients lack p16 staining and were not included in the analysis, many of them from the early years of the study and their outcome was similar to the p16-negative patients. The exclusion of these patients' group may impact the findings of the current study, however, assuming many of these patients were p16-negative (due to similar survival), the trends of increasing p16-positivity over the years in north Israel is enhanced.

In addition, other potential risk factors could not be fully assessed due to incomplete documentation in the medical records. Characteristics of the population, including the high proportion of immigrants and the representation of various ethnicities, may have influenced the proportion of p16-positivity. The higher proportion of p16-negative OPSCC among the Arabs than the Jews may be related to more conservative attitudes and more prevalent smoking in the Arab sector (as for 2024, 23.3% of the Arab sector smoke, compared to 19.9% in the Jewish sector. Furthermore, 40.3% of the Arab males smoke, as compared to 23% of the Jewish males) [26]. However, the retrospective design precluded examining either of these aspects.

The current study did not evaluate changes in the age-standardized incidence rate of OPSCC during the last decade in northern Israel. Although Rambam Health Care Campus is the sole comprehensive head and neck oncology center for northern Israel, patients may be treated in other centers, and therefore the number of patients is not standardized to the population number. During the study years, the AJCC staging system changed from that described in the 7^{th} to the 8th Edition. Re-staging of all the patients according to the AJCC 8^{th} Edition down staged patients with p16-positive OPSCC. Therefore, differences in the staging between p16-positive and HPV-negative patients may be related to the new staging system. Furthermore, the treatment protocol changed during the study period, which may also have influenced outcomes.

Conclusions

In this study of the evolving epidemiology of p16-related OPSCC in northern Israel during 2012-2024, the proportion of OPSCC that was p16-positive, as assessed by p16 IHC, accounted for 72%. This represents a trend of substantial increase over the 34% proportion reported for Israel overall in 2011 [13]. In the latter years of the study period, the proportion rose further to 78%, thus mirroring trends observed in North America and Northern Europe. Notably, in 2023–2024, p16-positivity OPSCC in northern Israel rose sharply, and accounted for 92% of all OPSCC diagnoses. Using a combined parameter of p16 status and T stage, p16-negative and advanced T stage was the most significant predictor for poorer overall survival, as was treatment with concurrent cetuximab, which also represents more advanced disease.

Abbreviations

AJCC, American Joint Committee on Cancer

CAP, College of American Pathologists

CI, confidence interval

DHANCA, Danish Head and Neck Cancer group radiotherapy protocol

HPV, human papillomavirus

HR, hazard ratio

HR-HPV, high-risk HPV

IHC, immunohistochemistry

IMRT, intensity-modulated radiotherapy

OPSCC, oropharyngeal squamous cell carcinoma

RT, radiotherapy

Declarations

Ethics approval: The study approved by the Institutional Review Board of Rambam Health Care Campus (0838-20-RMB, 27-12-2023).

Informed Consent Statement: Patient's consent was waived due to the retrospective nature of the study as the study involved the analysis of existing electronic medical records, posed no risk to the patients, and used de-identified data**.**

Consent for publication: Patient's consent was waived due to the retrospective nature of the study.

Data Availability Statement: The data that support the findings of this study are available from the Rambam Health Care Campus; however, institutional restrictions apply to the availability of these data. Requests regarding data verification or accuracy should be directed to the corresponding author (idoweck@technion.ac.il).

Conflicts of Interest All the authors declared no conflicts of interest related to this work.

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Author Contributions

SBF: Conceptualization, Data curation, Formal analysis, Methodology, Investigation, Writing – original draft, Writing – review & editing; **SS:** Writing – review & editing; **NS:** Data curation, Formal analysis, Writing – review & editing; **ES:** Writing – original draft, Writing – review & editing; **SB:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing; **ID:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. All authors read and approved the final manuscript.

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Figures

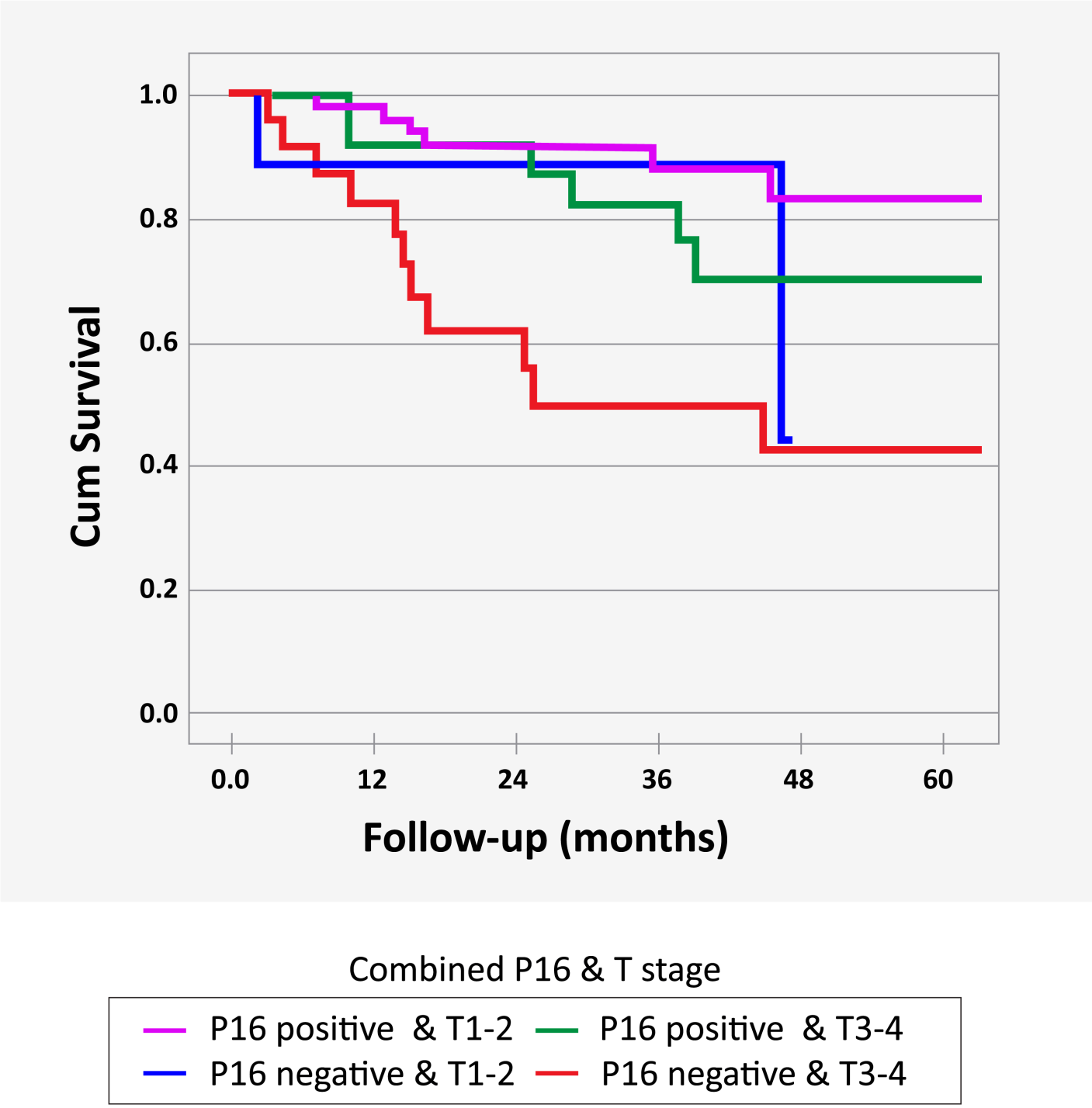


Figure 1

Overall survival stratified by combining p16 status and T stage: p16-positive and early T stage, p16-positive and advanced T stage, p16-negative and early T stage (biased due to the small number of patients) and p16-negative and advanced T stage