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The Impact of HPV Infection on the Development of Oropharyngeal Cancer: A Single-Center Experience

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Rezumat

Impactul infecției cu HPV asupra dezvoltării cancerului orofaringian: experiența unui singur centru

Introducere: orofaringele a fost identificat drept localizarea anatomică predominantă a carcinomului cu celule scuamoase HPV-pozitiv, afecțiune strâns asociată cu factori de risc precum consumul de tutun şi alcool. Totuşi, în ultimele 2-3 decenii, s-a observat o creştere a cazurilor asociate infecției HPV.
Material şi metode: Am analizat o bază de date cuprinzând 53 de pacienți care s-au prezentat în clinica noastră cu cancer orofaringian şi status HPV necunoscut, în ultimii doi ani. Analiza statistică a fost efectuată utilizând instrumente analitice asistate de inteligența artificială.
Rezultate: Acest raport prezintă o analiză statistică a 53 de cazuri. Setul de date cuprinde o variabilă continua (vârsta) şi 5 variabile categorice cheie de referință (sexul, stadiul bolii, statusul HPV, statusul privind fumatul şi consumul de alcool). Rezultatele sugerează că variabilele de referință masurate sunt indepedente şi vârsta nu prezintă o asociere evidentă cu niciunul dintre factorii de referință luați individual.
Concluzii: Populația studiată a fost formată din persoane de vârstă mijlocie şi vârstnici, cu o medie de aproximativ 63 de ani. Factorii de risc precum consumul de tutun şi alcool rămân frecvenți în rândul pacienților cu cancer orofaringian, inclusiv în cazul celor cu tumori HPV-pozitive.

Cuvinte cheie: statusul HPV, cancer orofaringian, carcinom cu celule scuamoase

Abstract

Introduction: The oropharynx has been consistently identified as the predominant anatomical site for HPV-positive squamous cell carcinoma, which has been strongly associated with risk factors such as tobacco use and excessive alcohol consumption. However, over the past 20-30 years, a significant increase in cases associated with

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infection with HPV has been observed.
Material and Methods: We analyzed a database comprising 53 patients who presented to our clinic with oropharyngeal cancer and unknown HPV status over the past two years. Statistical analysis was performed using SPSS and artificial intelligence-assisted analytical tools.
Results: This report provides a statistical review of 53 patient records. The dataset contains one continuous variable (Age) and five key categorical baseline variables (Sex, Stage, HPV Status, Smoking Status, and Alcohol Consumption). The results suggest the measured baseline variables are largely independent and that age is not obviously confounded with any single baseline factor.
Conclusions: The study population consisted mainly of middle-aged to elderly individuals, with a mean age of around 63 years. Risk factors such as tobacco use and alcohol consumption remain common among patients with OPC, even among those with HPV-positive tumors.

Keywords: HPV status, oropharyngeal cancer, squamous cell carcinoma

Introduction

Oropharyngeal cancer has become an increasingly important global health problem. In the United States of America, it accounts for approximately 3% of all malignancies. The oropharyngeal cancer includes subsites such as the base of the tongue, the palatine tonsils, the soft palate, as well as the lateral and posterior pharyngeal walls. The oropharynx has been consistently identified as the predominant anatomical site for HPV-positive squamous cell carcinoma, while involvement of other head and neck subsites is reported far less frequently in the literature. Consequently, the present study primarily focuses on patients diagnosed with oropharyngeal carcinoma, although HPV-associated lesions at other head and neck locations were also identified during the data collection period (1-4).

Traditionally, the development of oropharyngeal squamous cell carcinoma has been strongly associated with well-known risk factors such as tobacco use and excessive alcohol consumption.However, over the past 20-30 years, a significant increase in cases associated with infection with human papillomavirus (HPV) has been observed, particularly with the high-risk subtype Human papillomavirus 16 (3-11).

The growing prevalence of HPV-related oropharyngeal malignancies represents a major public health concern. Notably, this trend has led to the emergence of the so-called "atypical patient": younger individuals without traditional risk factors such as smoking or chronic alcohol consumption. This epidemiological shift highlights the need for increased awareness, preventive strategies - including HPV vaccination - and tailored therapeutic approaches (12-14).

Most HPV infections are acquired through sexual contact with an infected partner. Given the high prevalence of cervical HPV infection and the significant risk of malignant transformation associated with cervical condylomatous lesions, HPV screening through co-testing and genotyping remains a fundamental component of routine gynecological surveillance and is recommended during annual gynecological examinations. Furthermore, recent guidelines and emerging evidence advocate for otorhinolaryngological (ENT) evaluation in both partners when HPV-related lesions are detected or when one partner tests positive for HPV, due to the potential involvement of the upper aerodigestive tract in HPV-associated disease (15-17).

Following the introduction of the quadrivalent HPV vaccine and, subsequently, the nonavalent formulation, numerous countries have incorporated HPV vaccination into national immunization programs and broader public health strategies, which has been associated with a measurable decline in HPV prevalence. However, the cost-effectiveness and large-scale implementation of the nonavalent vaccine, particularly when compared with earlier formulations such as the bivalent and quadrivalent vaccines, remain subjects of ongoing debate in the scientific literature (18,19). Moreover, substantial evidence demonstrates a reduction in the incidence of cervical cancer following the implementation of HPV vaccination programs. However, the relationship between HPV vaccination and the incidence of oropharyngeal cancer remains less clearly established, particularly among men, and continues to be an area of ongoing investigation (20). From another perspective, analyses of social media content addressing the dissemination of information on HPV vaccination - particularly regarding its efficacy, potential adverse effects, and eligibility criteria—indicate that public discourse predominantly emphasizes its protective effect against

cervical cancer. This focus often overlooks awareness of HPV-associated malignancies at other anatomical sites and underrepresents the impact of HPV infection and vaccination in the male population (21-24).

Material and Methods

For this study, we analyzed a database comprising 53 patients who presented to our clinic with oropharyngeal cancer and unknown HPV status over the past two years.

The inclusion criteria consisted of patients diagnosed with stage I through IV oropharyngeal cancer who had not received any prior treatment, including radiotherapy, chemotherapy or surgery.

The exclusion criteria included patients who were unfit for surgical management, such as those with T4b stage tumors, M1 stage disease, or significant comorbidities that precluded a surgical approach.

Statistical analysis was performed using SPSS and artificial intelligence–assisted analytical tools.

Results

After collecting cases from the past two years, the data were organized into a table including age, sex, and known risk factors such as tobacco use and alcohol consumption.

Polymerase chain reaction (PCR) testing was performed on biopsy specimens to confirm the presence of viral DNA. The next steps after the DNA extraction were PCR target selection, amplification, detection and genotyping. Since the mere presence of HPV DNA does not always demonstrate a direct causal relationship with the tumor, immunohistochemistry for the p16 protein was additionally performed. By correlating these two analyses, the diagnosis of HPV-induced oropharyngeal carcinoma was confirmed with certainty.

The results are presented in *Table 1.*

This report provides a comprehensive statistical review of 53 patient records. The dataset contains one continuous variable (Age) and five key categorical

Table 1. Data gathered for the study

Patient_ID	Sex	Age	Stage	HPV Status	Smoking Status	Alcohol Consumption
P001	M	69	1	Negative	Non-smoker	None
P002	M	50	1	Negative	Smoker	>2 units/day
P003	M	80	2	Positive	Non-smoker	>2 units/day
P004	M	63	3	Positive	Non-smoker	None
P005	M	68	2	Negative	Smoker	None
P006	F	81	2	Negative	Non-smoker	None
P007	M	57	3	Negative	Smoker	None
P008	F	49	3	Negative	Smoker	<2 units/day
P009	M	47	1	Positive	Smoker	<2 units/day
P010	M	59	1	Negative	Non-smoker	<2 units/day
P011	F	63	2	Negative	Smoker	None
P012	M	50	3	Negative	Non-smoker	<2 units/day
P013	F	59	2	Negative	Smoker	None
P014	F	51	2	Positive	Smoker	None
P015	F	69	3	Positive	Non-smoker	<2 units/day
P016	F	62	3	Positive	Smoker	None
P017	M	74	4	Positive	Smoker	<2 units/day
P018	F	68	1	Negative	Smoker	<2 units/day
P019	M	55	4	Negative	Smoker	<2 units/day
P020	F	68	1	Negative	Smoker	<2 units/day
P021	F	67	4	Negative	Smoker	<2 units/day
P022	M	58	4	Positive	Smoker	>2 units/day
P023	F	62	4	Positive	Smoker	>2 units/day
P024	M	49	1	Negative	Non-smoker	>2 units/day
P025	F	83	3	Positive	Smoker	>2 units/day
P026	F	55	3	Negative	Smoker	>2 units/day
P027	F	79	2	Negative	Smoker	<2 units/day
P028	F	60	4	Positive	Non-smoker	None
P029	F	55	4	Negative	Non-smoker	None

Table 1. Cont'd

Patient_ID	Sex	Age	Stage	HPV Status	Smoking Status	Alcohol Consumption
P030	M	74	1	Positive	Smoker	<2 units/day
P031	M	69	3	Negative	Smoker	None
P032	M	62	1	Positive	Smoker	None
P033	F	80	3	Positive	Smoker	>2 units/day
P034	F	59	4	Negative	Non-smoker	>2 units/day
P035	F	65	3	Negative	Smoker	>2 units/day
P036	M	48	3	Positive	Smoker	>2 units/day
P037	F	59	3	Negative	Smoker	>2 units/day
P038	F	47	3	Negative	Smoker	<2 units/day
P039	M	65	3	Positive	Smoker	None
P040	F	70	2	Positive	Smoker	<2 units/day
P041	F	62	2	Positive	Smoker	>2 units/day
P042	M	49	3	Positive	Smoker	<2 units/day
P043	F	58	3	Positive	Smoker	>2 units/day
P044	F	81	1	Positive	Non-smoker	>2 units/day
P045	M	65	1	Negative	Smoker	None
P046	F	58	4	Positive	Smoker	>2 units/day
P047	M	76	1	Negative	Non-smoker	>2 units/day
P048	M	70	2	Positive	Smoker	None
P049	M	74	4	Negative	Smoker	None
P050	F	54	3	Negative	Non-smoker	None
P051	M	61	2	Negative	Non-smoker	>2 units/day
P052	M	53	2	Positive	Smoker	None
P053	F	60	4	Negative	Smoker	>2 units/day

baseline variables (Sex, Stage, HPV Status, Smoking Status, and Alcohol Consumption).

The main quantitative finding is that age was approximately normally distributed (Shapiro-Wilk W = 0.962, p = 0.089),mean age was 62.8 years (SD = 9.9) and median 62.0 years (IQR 55.0-69.0).

The *Figs. 1-9* illustrate the distribution of the study subjects according to the variables collected.

The age distribution was approximately normal (Shapiro-Wilk p = 0.089). Observed age values tracked the expected normal quantiles reasonably well, with no major departure from normality.

None of the subgroup comparisons reached the conventional threshold for statistical significance ($p < 0.05$). Moreover, the observed effect sizes were consistently small, supporting the conclusion that age

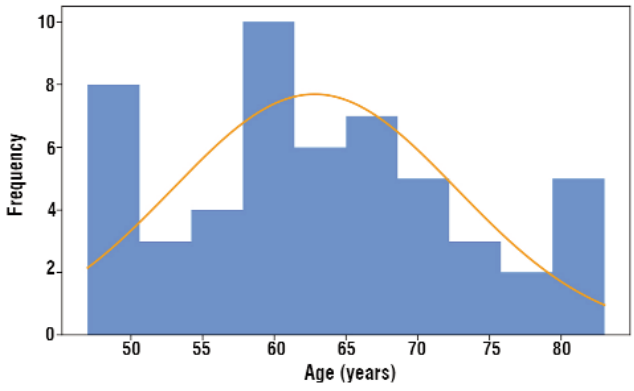


Figure 1. Age distribution

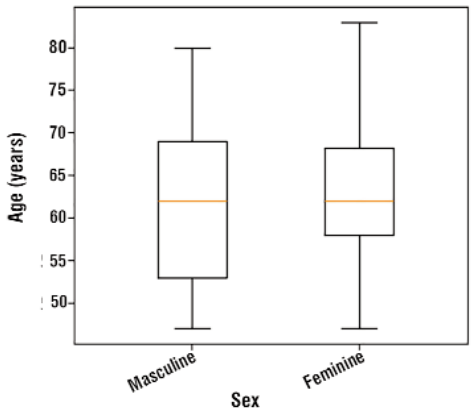


Figure 2. Age by Sex

Table 2. Summary of within-cohort associations

Grouping variable	Levels	Mean age by level	Welch t p	Mann-Whitney p	Effect size	Effect size type	ANOVA p	Kruskal-Wallis p
Sex	Masculine vs Feminine	61.8 vs 63.7	0.488	0.599	-0.192	Cohen d		
Stage	1, 2, 3, 4	1=64.0; 2=66.4; 3=60.1; 4=62.0			0.061	Eta squared	0.374	0.306
HPV_Status	Negative vs Positive	61.7 vs 64.1	0.392	0.406	-0.242	Cohen d		
Smoking_Status	Non-smoker vs Smoker	64.4 vs 62.2	0.501	0.547	0.223	Cohen d		
Alcohol_Consumption	Missing, >2 units/day, <2 units/day	Missing=63.2; >2 units/day=63.4; <2 units/day=61.7			0.005	Eta squared	0.872	0.862

differences across these baseline groups were minimal within the present sample.

Visual inspection suggests modest differences between groups; however, the formal statistical analysis did not demonstrate statistical significance (p = 0.488).

Visual comparison suggests modest between-group differences, but the formal test was not statistically significant (p = 0.374).

Visual comparison suggests modest between-group differences, but the formal test was not statistically significant (p = 0.392).

Visual comparison suggests modest between-group differences, but the formal test was not statistically significant (p = 0.501).

Visual comparison suggests modest between-group differences, but the formal test was not statistically significant (p = 0.872).

Confidence intervals overlapped substantially across stages, consistent with the non-significant ANOVA and Kruskal-Wallis results.

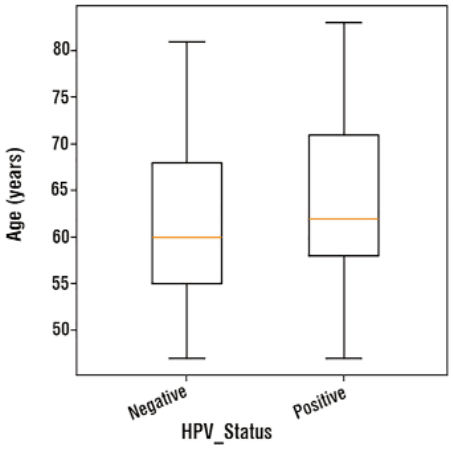


Figure 3. Age by Stage

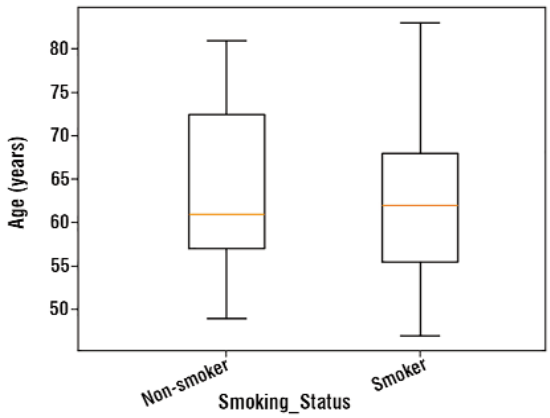


Figure 4. Age by Smoking status

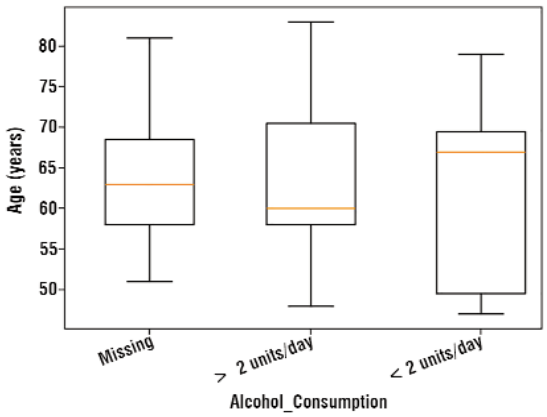


Figure 5. Age by Alcohol consumption

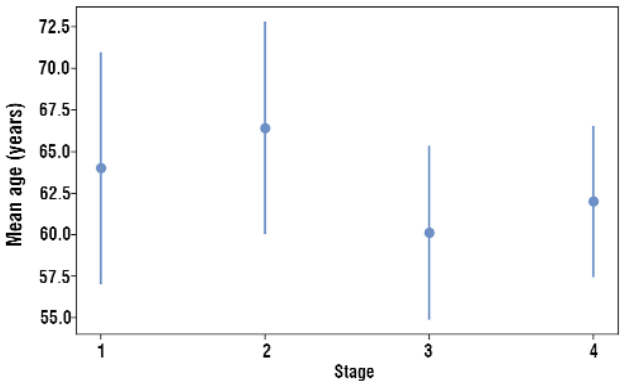


Figure 6. Mean age with 95% CI by stage

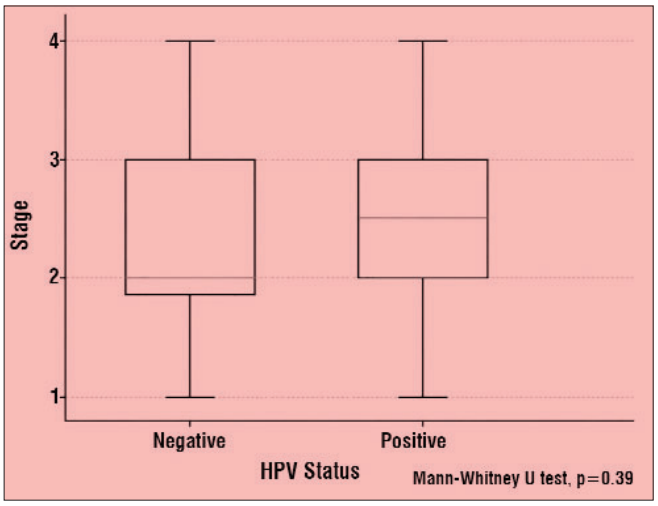


Figure 7. Stage by HPV status

None of the subgroup comparisons met the 0.05 significance threshold. Effect sizes were small throughout, supporting the conclusion that age differences across these baseline groups were limited in this sample.

There was no statistically significant difference in stage distribution between HPV-positive and HPV-negative patients (p=0,96).

HPV positivity was higher in smokers (50.0%) than in non-smokers (33.3%), but this difference was not statistically significant.

HPV positivity was 42.1% in the "None" category, 40.0% with <2 units/day, and 52.6% with >2 units/day; the overall difference was not significant.

The HPV-positive proportions were comparable in

Table 3. Statistical indicators regarding the association of variables

Variables	Chi-square p	Fisher exact p	Cramer V
Sex vs Stage	0.180		0.304
Sex vs HPV_Status	0.921	0.786	0.014
Sex vs Smoking_Status	0.795	0.761	0.036
Sex vs Alcohol_Consumption	0.429		0.179
Stage vs HPV_Status	0.811		0.135
Stage vs Smoking_Status	0.693		0.166
Stage vs Alcohol_Consumption	0.619		0.204
HPV_Status vs Smoking_Status	0.429	0.363	0.109
HPV_Status vs Alcohol_Consumption	0.719		0.112
Smoking_Status vs Alcohol_Consumption	0.701		0.116

Figure 8. HPV status and smoking

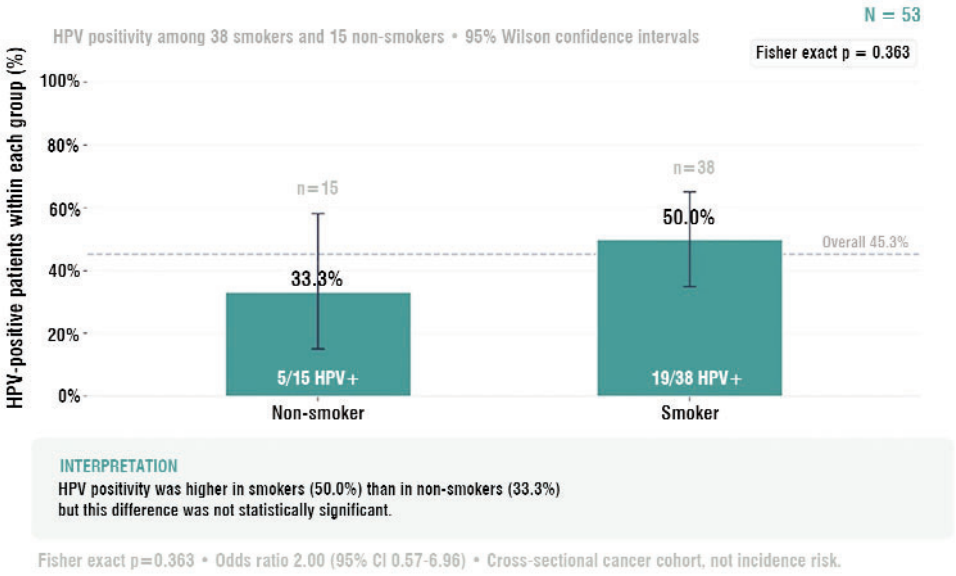
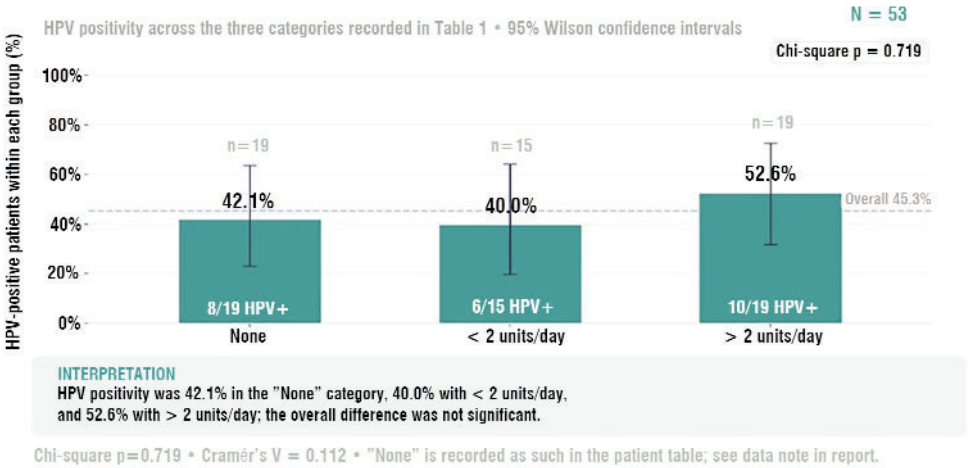


Figure 9. HPV status and alcohol consumption



women (42.9%) and men (48.0%). The study did not identify evidence of an association with sex.

Overall Results

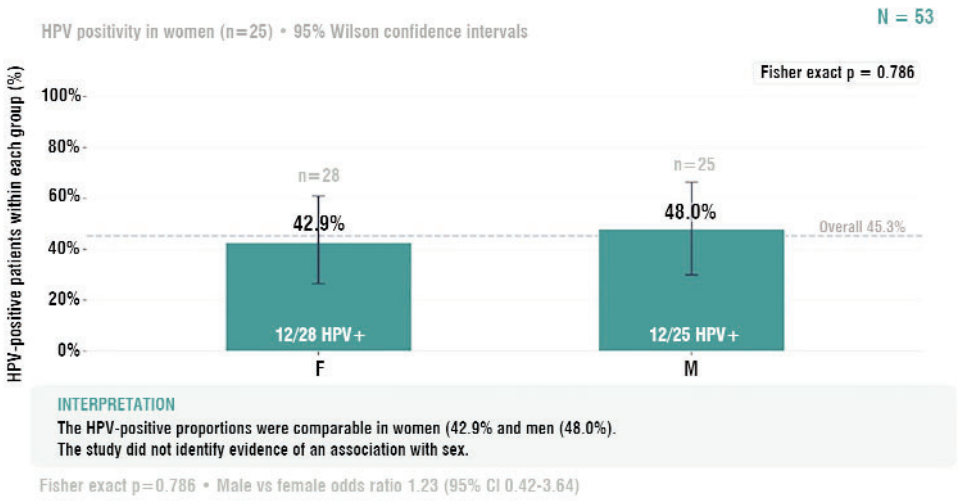
All observed Cramer's V values were in the weak range, and none of the tested relationships reached statistical significance. Several larger contingency tables had expected cell counts below 5, which reduces the reliability of asymptotic chi-square p-values and argues for cautious interpretation.

The dataset characterizes baseline patient demographics rather than treatment outcomes. Participants are middle-aged to older adults with a well-behaved, roughly symmetric age distribution and no large age imbalances across groups. Across recorded baseline factors there is no clear evidence of major imbalances or strong dependencies among categorical variables. Overall, these results suggest the measured baseline variables are largely independent and that age is not obviously confounded with any single baseline factor. However, the sample size is modest and several contingency tables are sparse, so modest true associations could have been missed.

Discussion

To expand this analysis into a full clinical or trial-based study, additional variables should be incorporated, includingrandomized treatment groups, treatment exposure, treatment response, recurrence status, survival time, censoring status, and adverse events. With these variables available, the analysis could be extended to include assessment of baseline balance

Figure 10. HPV status and sex



between treatment arms, logistic regression models for binary outcomes, Kaplan–Meier survival analysis, Cox proportional hazards regression, and multivariable adjusted outcome models.

Furthermore, this study highlights the need for a larger database and an extended follow-up period. Expanding the sample size and study duration would allow for more reliable estimation of outcomes such as survival rates, recurrence rates, and the identification of additional clinically significant prognostic factors.

Limitations

First, the dataset does not include treatment assignment, follow-up time, response, toxicity, or survival endpoints. Some patients received treatment at local oncology services. Consequently, data regarding their treatment and survival were incomplete and the current analysis cannot evaluate efficacy, prognosis, time-to-event outcomes, or baseline balance across treatment groups.

Second, alcohol-consumption data are missing for more than one-third of participants, which reduces precision in alcohol-related subgroup analyses.

Third, small cell counts in some multi-level contingency tables decrease the power and stability of chi-square-based inference.

Finally, given the relatively small sample size, non-significant findings should not be interpreted as evidence of no association.

Conclusions

1. HPV infection represents an important etiological factor in oropharyngeal cancer, being identified in a significant proportion of the patients included in this cohort.
2. The study population consisted mainly of middle-aged to elderly individuals, with an approximately normal age distribution and a mean age of around 63 years.
3. No statistically significant associations were identified between age and the evaluated baseline variables, including sex, tumor stage, HPV status, smoking status, and alcohol consumption, given the relatively small cohort.
4. Traditional risk factors such as tobacco use and alcohol consumption remain common among patients with oropharyngeal cancer, even among those with HPV-positive tumors.

Conflicts of Interest

Nothing to declare.

Ethical Approval

Data collection regardind the patient cohort was approved by the Ethics Committee of Colțea Clinical Hospital (approval no. 9127, 23 May 2022).

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