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CORRELATION PD-L1 AND P53 PROTEINS WITH HUMAN PAPILLOMAVIRUS STATUS IN OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

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ABSTRACT

Background: In addition to chemical carcinogens, an important role is played by the human papillomavirus (HPV), whose prevalence is steadily growing and becoming an epidemic. According to Centers for Disease Control and Prevention about 70% of cases of oropharyngeal squamous cell carcinoma (OPSCC) are caused by HPV. HPV+OPSCC has specific biological and immunological properties and has a significantly better response to treatment and higher overall survival compared. **Methods:** The study included 62 patients treated with OPSCC T1-4N0-3M0 (7th edition, AJCC) in 2015-2020 in clinics located in two large cities of Uzbekistan (Tashkent and Samarkand). All patients (n=62) underwent IHC analysis for $p16^{INK4a}$, PD-L1 and p53 proteins in formalin-fixed paraffin embedding tumor tissue samples. The $p16^{INK4a}$ IHC was the only HPV status test that was interpreted according to the recommendations of the College of American Pathologists. There is a subgroup of patients with HPV+OPSCC with a tendency to relapse, which leads to discrimination of disease outcomes. **Results:** Positive expression of PD-L1 improved overall survival compared to negative expression (p = 0.261) in HPV-positive OPSCC (p=0.261). In all cases, a negative correlation was observed between p53mutant and HPV status (p<0.001). Only in the HPV-negative group did negative expression of p53mutant lead to a slight prolongation of patients' lives compared to positive expression (p<0.001). **Conclusion:** Integration of HPV status with other molecular markers and risk factors may help reveal the unique clinical and molecular characteristics of OPSCC.

KEYWORDS: oropharyngeal squamous cell carcinoma, human papillomavirus, p16, the programmed death-ligand 1 (PD-L1), p53, immunohistochemistry.

BACKGROUND

Chronic exposure to tobacco smoking along with alcohol use is the most common cause of head and neck squamous cell carcinoma.[1] In addition to these carcinogens, an important role is played by the human papillomavirus (HPV), whose prevalence is steadily growing and becoming an epidemic. Scientific reports suggest that over the past two decades there has been a dramatic increase in the number of patients with HPV-positive oropharyngeal squamous cell carcinoma (HPV+OPSCC). In studies The Centers for Disease Control and Prevention reports that about 70% of cases of OPSCC are caused by persistent HPV infection.[2] In 2020, there were 98,412 cases of oropharyngeal cancer in the world, and the death rate from this disease was 48,143 people.[3] The most common, economical and sensitive methods for detecting HPV are the immunohistochemical determination of the p16 protein.[4] HPV+OPSCC has specific biological and immunological properties and has a significantly better response to treatment and higher overall survival compared to HPV-negative OPSCC (HPV−OPSCC).[5,6] A number of randomized trials have explored the promise of a deintensification strategy to reduce the toxic effects of treatment without compromising oncological outcomes.[7] There is a subgroup of patients with HPV+OPSCC with a tendency to relapse, which leads to discrimination of disease outcomes. The most significant barrier to identifying patients at high risk of relapse is the lack of clinically useful molecular predictors. Based on these data, the purpose of our study is the immunohistochemical measurement of the expression of programmed death-ligand 1 (PD-L1) and p53 proteins,

the study of the influence of the level of expression of these proteins on overall survival of patients with OPSCC, depending on HPV status.

METHODS

Our retrospective study included 62 patients treated with OPSCC T1-4N0-3M0 (7th edition of the American Joint Committee on Cancer, AJCC) in 2015-2020 at the Republican specialized scientific and practical medical center for oncology and radiology and its filial branches located in two large cities of Uzbekistan (Tashkent and Samarkand). Criteria for inclusion in the study: histologically confirmed squamous cell carcinoma, tumors located only in the oropharynx (palatine tonsil, base of the tongue, soft palate, lateral wall, posterior wall), absence of distant metastases at diagnosis, treated patients, patients having archival histological material that meets the requirements of immunohistochemistry and patients over 18 years of age. All medical records were reviewed to determine patient demographics (gender, age, smoking and alcohol, ECOG status, disease course, treatment details, outcome) and tumor characteristics (TNM stage, tumor location, histology) at diagnosis. A database of all collected cases of OPSCC was created using departmental cancer registries and medical records of patients in accordance with the methodological requirement. The present study was approved by the protocol decision of the scientific council of the institution, since archived tumor material was used for immunohistochemical analysis in accordance with local ethical requirements. All retrospectively collected patients (n=62) underwent immunohistochemical analysis for p16, PD-L1 and p53 proteins in formalin-fixed paraffin embedding (FFPE) tumor tissue samples.

In the present study, immunohistochemistry (IHC) of the p16 protein was the only test to establish HPV status in patients with OPSCC, which was interpreted according to the guidelines of the College of American Pathologists.[8] The absence and local staining of p16 or the presence of <70% of stained tumor cells in the patterns were considered HPV-negative (Fig.1a), if ≥70% of tumor cells showed strong and diffuse nuclear and cytoplasmic staining, then they were considered HPV-positive (Fig.1b). To determine the expression level of the p16 protein, the CINtec® Histology IHC test was used in accordance with the manufacturer's instructions (Ventana Medical Systems, Inc., Tucson, AZ, USA) containing a primary mouse monoclonal antibody of clone E6H4, which is optimized for use on the Ventana BenchMark system in combination with the OptiView DAB IHC Detection Kit.

PD-L1 expression was assessed using monoclonal antibody clone SP263 (Ventana Medical Systems, Inc., Tucson, AZ, USA) on a Ventana BenchMark automatic immunohistotainer according to standard protocols in FFPE samples. The expression level of PD-L1 was assessed by the proportion of positively stained tumor cells (TPS, tumor proportion score), demonstrating partial linear or complete circular staining of the membrane. The results of PD-L1 IHC were evaluated as positive if the stained tumor cells were TPS≥1%. According to the intensity of expression, PD-L1 were classified as negative 0-0.9% (PD-L1-N/negative), low TPS 1-9% (PD-L1-L), medium TPS 10-29% (PD-L1-M), high TPS 30-49% (PD-L1-H) and very high TPS ≥50% (PD-L1-VH) (Fig. 2). In addition, when determining the expression of the p53 protein in FFPE tumor samples, the monoclonal antibody of the Bp53-11 clone (Ventana Medical Systems, Inc., Tucson, AZ, USA) was used on the same Ventana BenchMark automated system for conducting immunohistochemical reactions. The primary antibody of clone Bp53-11 is directed against both the mutant type and the wild type of the p53 nuclear protein. The threshold for positive p53 expression was ≥10% nuclear staining, and other patterns were considered negative (Fig. 1c, d, e). Depending on the degree of expression intensity, it was classified into the following: negative 0-9% (p53mut-N/negative), low 10-29% (p53mut-L), medium 30-49% (p53mut-M), high 50-79% (p53mut-H) and very high ≥80% (p53mut-VH) and similar for p53wild. The many international protocols and consortiums that are currently operating in the context have been taken into account in developing the classification for the calibration of protein expression scores.

Statistical analysis was carried out using SPSS Statistics version 26.0 (IBM Corporation, Armonk, NY, USA) on a Windows 10 user operating system (Microsoft Corporation, Redmond, WA, USA). We used Pearson's correlation (Pearson's r-coefficient) to assess the relationship between protein expression (PD-L1, p53), other predictors (smoking, alcohol, age), and HPV status. For comparative analysis of mean values, Student's t-test was used for samples. The Kaplan-Meier method was used to describe overall survival, and differences between groups were tested for significance using a logrank test (Mantel-Cox). Overall survival (OS) was determined from the time of initial diagnosis to the date of death from any cause. A Cox proportional hazards regression model was used to assess the effect of PD-L1, p53, and the above predictors on OS. Also, the hazard ratio (HR) and 95% confidence interval (CI) were assessed. A *p* value of less than 0.05 was considered statistically significant.

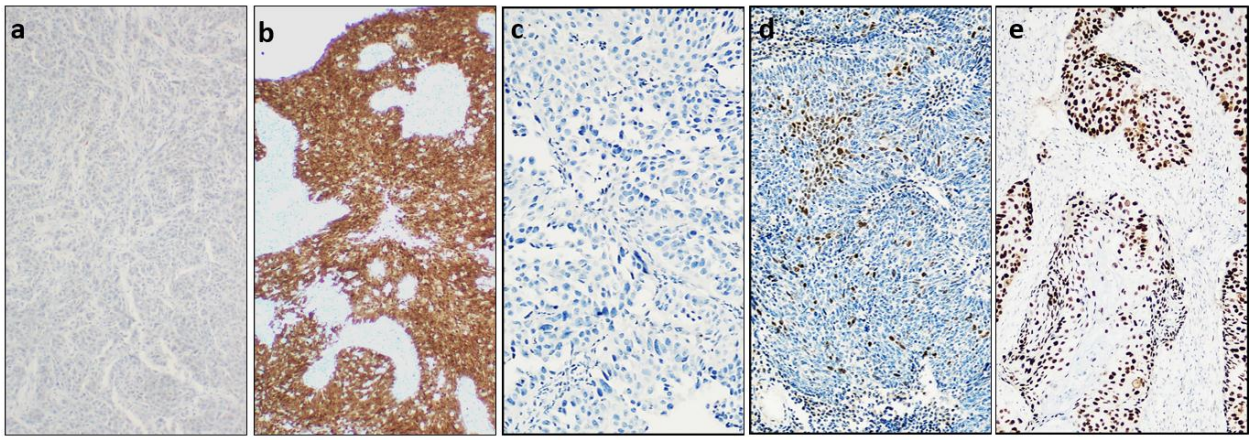


Figure 1: Expression of p16 and p53 proteins in OPSCC detected by IHC: a) negative expression of p16, b) strong diffuse expression of p16 (>70%), characteristic of HPV+ OPSCC, c) negative expression of p53, d) positive expression p53wild (low level), e) p53mutant positive expression (high level) (x100).

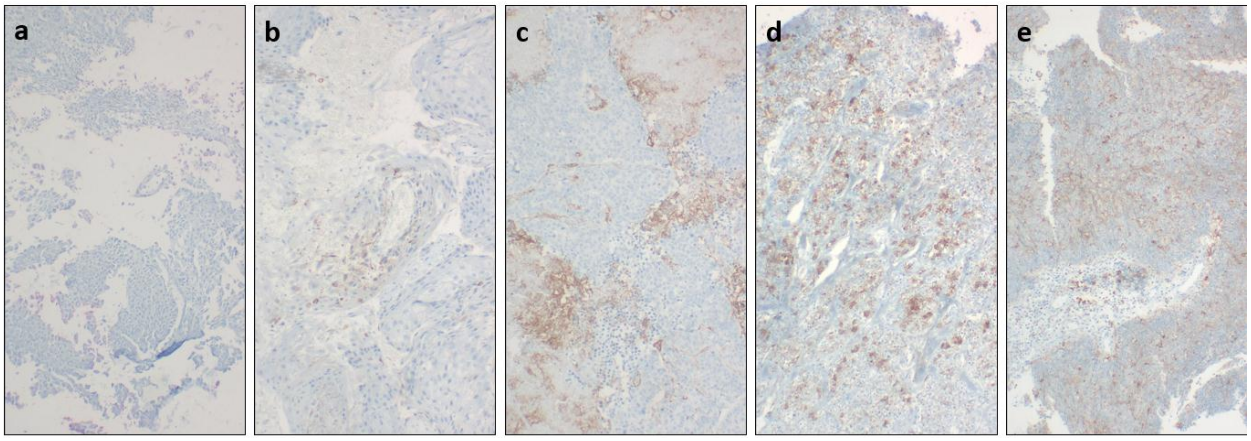


Figure 2: PD-L1 protein expression levels in tumor cells on IHC: a) negative expression, b) low level, c) medium level, d) high level, e) very high level. (x100)

RESULTS

Patients of the total group were divided into two subgroups according to p16 expression depending on positivity and negativity, accordingly. Clinical and demographic characteristics of patients and the overall frequency of expression of PD-L1, p53 proteins in the total group (n=62) and in groups divided by HPV status provided in the Table 1. The median follow-up was 41.8 months (2-107 months). In this study, the attributable fraction of HPV was 45.2%, that is 28 patients were registered as HPV+ OPSCC, and the remaining 34 (54.8%) were assessed as HPV− OPSCC. As for differences between groups, the mean age of patients in the HPV+ group is 47.3 years (interquartile range, IQR 24-77) and slightly lower compared to the total (53.8, IQR 24-79) and HPV− groups (59.2, IQR 34-79, $p<0.001$). Patients in the HPV+ group with statuses 0 and 1 according to ECOG have the highest rate (60.7%) compared with the total (46.7%) and HPV− (35.3%, $p=0.039$). Unlike other groups in 92.8% (26) patients in the HPV+ group, the tumor developed from the palatine tonsil and the base of the tongue ($p<0.001$). Compared to the total (37, 59.7%) and HPV− group (20, 58.8%), only 5 patients (17.9%) smoked in the HPV+ group ($p<0.001$). Alcohol consumption also has a similar difference, as in the HPV+ group 3 (10.7%) versus 15 (24.2%) in the total group and 12 (35.3%) in the HPV− group, which is statistically significant ($p=0.024$). As for the primary tumor, then in the HPV+ group, 50% conformed to the early stages of T1 and T2, and in the total (71%) and HPV− groups (88.2%), on the contrary, were identified advanced stages of T3 and T4 ($p<0.001$). In terms of N symbols, there was no evident difference between groups (p=0.747). Grouping by stages (TNMv7, AJCC) has a very sad scene most in HPV−, since 32 patients (94.1%) have advanced stages (III+IV), compared with HPV+ (24, 85.7%) and the total group (56, 90.3%, $p=0.061$).

Table 1: Clinical, demographic and pathological features of patients with OPSCC in groups.

Variables	Total (n=62), 100.0%	HPV status positive (n=28), 45.2%	HPV status negative (n=34), 54.8%	*p* value
Age Median, years (IQD*)	53.8 (24-79)	47.3 (24-77)	59.2 (34-79)	<0.001
Gender, №. (%) Male Female	35 (56.5) 27 (43.5)	16 (57.1) 12 (42.9)	19 (55.9) 15 (44.1)	0.922
ECOG, №. (%) 0 1 2 3	3 (4.8) 26 (41.9) 32 (51.6) 1 (1.6)	2 (7.1) 15 (53.6) 11 (39.3) 0	1 (2.9) 11 (32.4) 21 (61.8) 1 (2.9)	0.039
Tumour location, №. (%) Tonsils Base of tongue Soft palate Lateral wall Posterior wall	39 (62.9) 6 (9.7) 4 (6.5) 8 (12.9) 5 (8.1)	23 (82.1) 3 (10.7) 1 (3.6) 1 (3.6) 0	16 (47.1) 3 (8.8) 3 (8.8) 7 (20.6) 5 (14.7)	0.001
Smoking №. (%) Yes No	37 (59.7) 25 (40.3)	5 (17.9) 23 (82.1)	20 (58.8) 14 (41.2)	0.001
Alcohol №. (%) Yes No	15 (24.2) 47 (75.8)	3 (10.7) 25 (89.3)	12 (35.3) 22 (64.7)	0.024
T stage, №. (%) T1 T2 T3 T4	1 (1.6) 17 (27.4) 30 (48.4) 14 (22.6)	1 (3.6) 13 (46.4) 13 (46.4) 1 (3.6)	0 4 (11.8) 17 (50.0) 13 (38.2)	<0.001
N stage, №. (%) N0 N1 N2 N3	19 (30.6) 20 (32.3) 20 (32.3) 3 (4.8)	9 (32.1) 9 (32.1) 9 (32.1) 1 (3.6)	10 (29.4) 11 (32.4) 11 (32.4) 2 (5.9)	0.747
TNMv7, №. (%) 1 2 3 4	0 6 (9.7) 26 (41.9) 30 (48.4)	0 4 (14.3) 14 (50.0) 10 (35.7)	0 2 (5.9) 12 (35.3) 20 (58.8)	0.061
TNMv8, №. (%) 1 2 3 4	9 (14.5) 19 (30.6) 14 (22.6) 20 (32.3)	9 (32.1) 17 (60.7) 2 (7.1) 0	0 2 (5.9) 12 (35.3) 20 (58.8)	<0.001
PD-L1 №. (%) Positive Negative	37 (59.7) 25 (40.3)	17 (60.7) 11 (39.3)	20 (58.8) 14 (41.2)	0.882
p53mutant №. (%) Positive Negative	28 (45.2) 34 (54.8)	1 (3.6) 27 (96.4)	27 (79.4) 7 (20.6)	<0.001
p53wild №. (%) Positive Negative	5 (8.1) 57 (91.9)	5 (17.9) 23 (82.1)	0 34 (100.0)	0.01

* IQR, interquartile range.

Restaging according to the TNMv8 classification (AJCC, 2018) led to an increase in the proportion of early stages (I+II) by almost 5 times in the total group and amounted to 45.1%, and in the HPV+ group by 6.5 times and amounted an excellent rate of 92.8%, unfortunately, no changes in the HPV− group (p<0.001).

The IHC results revealed that more than half of the patients in the total group (37, 59.7%) had a positive expression of PD-L1, while the rest (25, 40.3%) had a negative expression. Positive expression of the mutant type p53 (p53mutant) was detected in 28 (45.2%) patients, negative - in 34 (54.8%). Frequency of PD-L1 expression did not differ significantly between groups stratified by HPV status (p=0.882). In the HPV− group, p53mutant was positive in 27 (79.4%) patients and negative in 7 (20.6%) patients, and in the HPV+ group, p53mutant was identified as exceptional only in 1 case (3.6%, p<0.001). Positive expression of wild-type p53 (p53wild) was detected only in 5 (8.1%) patients in the total group, all these belong to the HPV+ group, in the HPV− group, the activity of this protein is not identified (p=0.01). Distribution of PD-L1 expression levels in the total group: 13 (21%) PD-L1-L, 6 (9.7%) PD-L1-M, 9 (14.5%) PD-L1-H, 9 (14.5%) PD-L1-VH and 25 (40.3%) PD-L1-N. Compilation of this value in HPV+ patients: 7 (25%) PD-L1-L, 5 (17.9%) PD-L1-M, 4 (14.3%) PD-L1-H, 1 (3.57%) PD-L1-VH and 11 (39.2%) PD-L1-N, vs. 6 (17.7%), 1 (2.9%), 5 (14.7%), 8 (23.6%) and 14 (41.1%) in HPV− patients. The results of the analysis showed that PD-L1-H and PD-L1-VH were mainly found in the HPV− group, and PD-L1-L and PD-L1-M in the HPV+ group (p=0.254). The HR for PD-L1 in the overall study group was HR=1.082 (95% CI 0.390-3.002), in the HPV+ group HR=0.958 (95% CI 0.545-1.683) and in the HPV− group HR=1.036 (95% CI 0.656-1.636). Different degrees of expression of p53mutant were distributed as follows: 6 (9.7%) p53mut-L, 3 (4.8%) p53mut-M, 12 (19.4%) p53mut-H, 7 (11.3%) p53mut-VH and 34 (54.8%) p53mut-N. Almost all (27, 96.4%) patterns with positive expression of p53mutant belong to the HPV− group, since most of them had p53mut-H (12, 35.29%) and p53mut-VH (7, 20.59%) (p<0.001). Of the 5 (8.1%) p53wild positive, 4 (6.5%) were p53wild-L and 1 (1.6%) p53wild-M, all of them belong to the HPV+ group, on the contrary, p53wild was not identified in the HPV− group (p=0.015). The HR for the p53mutant protein in the total study group is HR=0.010 (95% CI 0.001-0.083), in the HPV− group HR=0.214 (95% CI 0.110-0.415), and in HPV+ group HR=22.235 (95% CI 3.22-153.534). This value for p53wild protein in HPV+ group is HR=0.404 (95% CI 0.294-0.553).

The Pearson correlation coefficient (r) was used to assess the relationship between predictors (PD-L1, p53mutant, p53wild, smoking, alcohol, age) and HPV status. According to the results of the assessment, it was revealed that there was practically no significant relationship between HPV status and PD-L1 (r=0.019, p=0.882), but there is a weak negative correlation with differential (levels) expression (r=−0.147, p=0.254). A very strong negative correlation with high statistical significance was found between p53mutant and HPV status (r=−0.758, p<0.001), and a moderate positive correlation between p53wild (r=0.326, p=0.01). There was a moderate negative correlation between HPV status and smoking (r=−0.416, p=0.001). A weak negative correlation coefficient was found between HPV status and alcohol (r=−0.286, p=0.024). Patient age has a moderate negative correlation with HPV status (r=−0.436, p<0.001). When correlations between predictors were analyzed without regard to HPV status, PD-L1 had a weak negative correlation with age alone (r=−0.147, p=0.255) and a weak positive correlation with all other variables. While p53mutant has a low negative correlation with p53wild (r=−0.269, p=0.035), a moderate positive correlation (r=0.509, p<0.001) with smoking, and a low positive correlation with alcohol (r=0.395, p=0.001) and age (r=0.311, p=0.014). In p53wild, a completely different pattern was found, which has a negative correlation with all factors but PD-L1. Smoking and alcohol, as recognized risk factors for cancer, have a moderate positive correlation between them (r=0.534, p<0.001).

The median overall survival (MOS) for males in the total group was 36 months (95% CI 14.2-57.8) versus 29 months for females (95% CI 12.1-45.9), however, by the end of the observation of the survival curve becomes slightly higher in the female population (p=0.092) (Fig.3a). The distribution of patients by HPV status resulted in a significant difference in OS between groups. OS in HPV+ patients are much improved (median not reached (MNR), after 24 months (ATFM)=96.4%) compared to HPV− (MOS=13 months [95% CI 8.9-17.1], ATFM=23.5%, p<0.001) (Fig.3b). In the total group of OS patients with positive PD-L1 is higher (MOS=46 months [95% CI NR], ATFM=60.5%) compared with PD-L1 negative (MOS=19 months [95% CI 0.0-38.6], ATFM=48%, p=0.364) (Fig.3c), p53mutant positive is lower (MOS=13 months [95% CI 9.3-16.7], ATFM=21.4%), vs p53mutant negative (MNR, ATFM=85.3%, p<0.001) (Fig.3d).

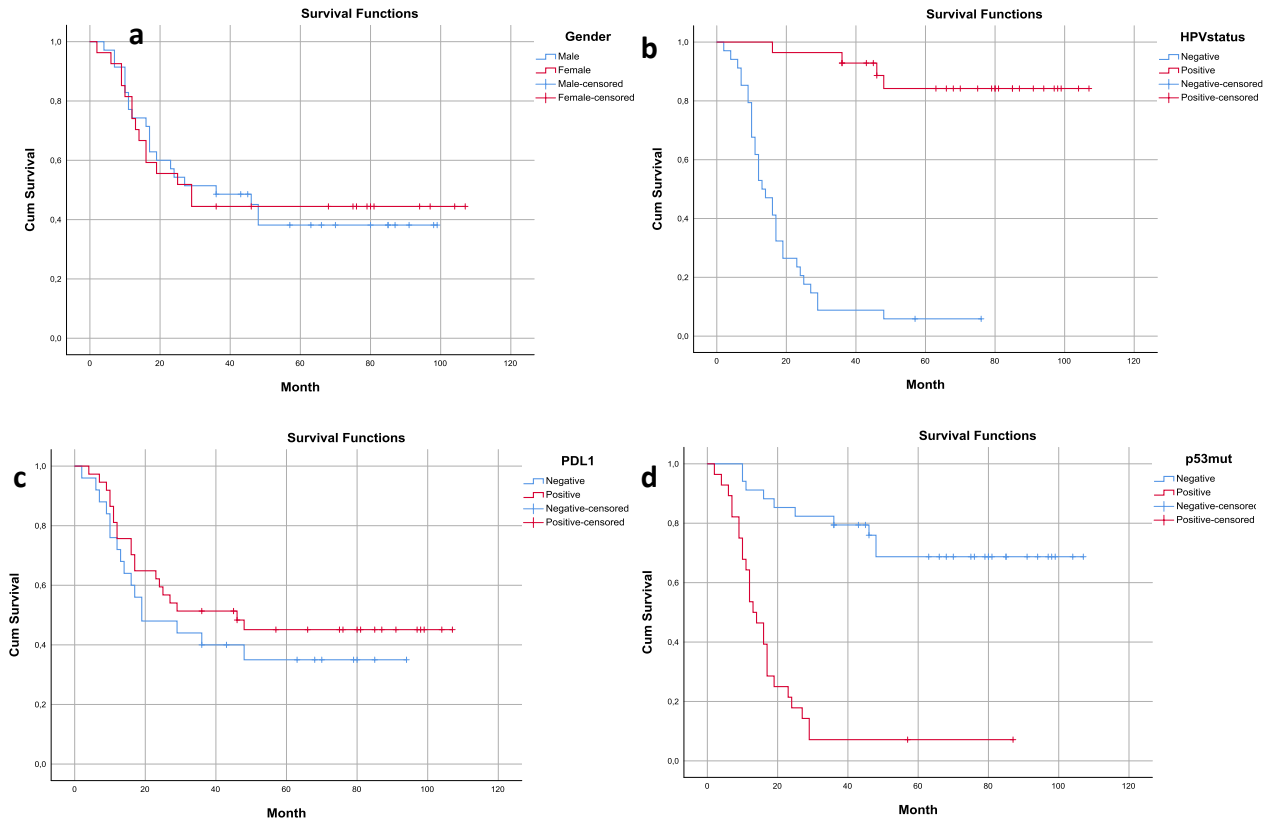
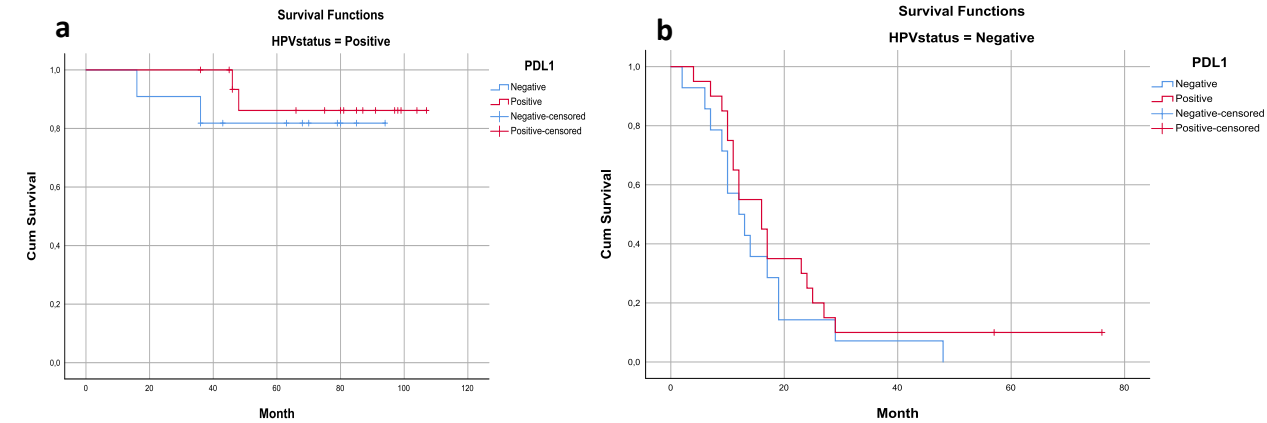


Figure 3: Overall survival of patients depending on gender (a), HPV status (b), expression PD-L1 (c) and p53mutant (d).

In the HPV+ group, PD-L1 negative expression had slightly worse survival rates (MNR, ATFM=90.9%) than positivity (MNR, ATFM=100%, *p*=0.261) (Fig.4a), there was no difference between negative p53mutant (MNR, ATFM=96%) and positive (MNR, ATFM=100%, *p*=0.229) (Fig.4c). In the HPV− group, too, negative expression of PD-L1 was worse result (MOS=12 months [95% CI 6.5-17.5], ATFM=14.3%) compared to positive (MOS=16 months [95% CI 7.3-24.7], ATFM=30%, *p*=0.261) (Fig.4b), a negative p53 mutant (MOS=19 months [95% CI 0.0-39.5], ATFM=42.9%) had a better survival compared to a positive p53 mutant (MOS=13 months [95% CI 9.9-16.1], ATFM=18.5%, *p*=0.229) (Fig.4d).

In addition, to study the dependence of survival time on independent variables and the assumption of predicting the risk of new events for the observed patients, the Cox regression method was used. The regression coefficient (RC) and HR were for HPV status (RC= -2.933, HR=0.053 [95% CI 0.018-0.156], *p*<0.001) compared with PD-L1 (RC= -0.300, HR=0.741 [95 % CI 0.384-1.431], *p*=0.372). The p53mutant was found to be the dominant risk factor with a negative impact on patient survival (RC=2.067, HR=7.901 [95% CI 3.653-17.090], *p*<0.001). Smoking (RC=1.047, HR=2.849 [95% CI 1.461–5.555], *p*=0.002) and alcohol (RC=0.797, HR=2.219 [95% CI 1.101–4.472], *p*=0.026) are also considered risk factors, which may increase the risk of death.



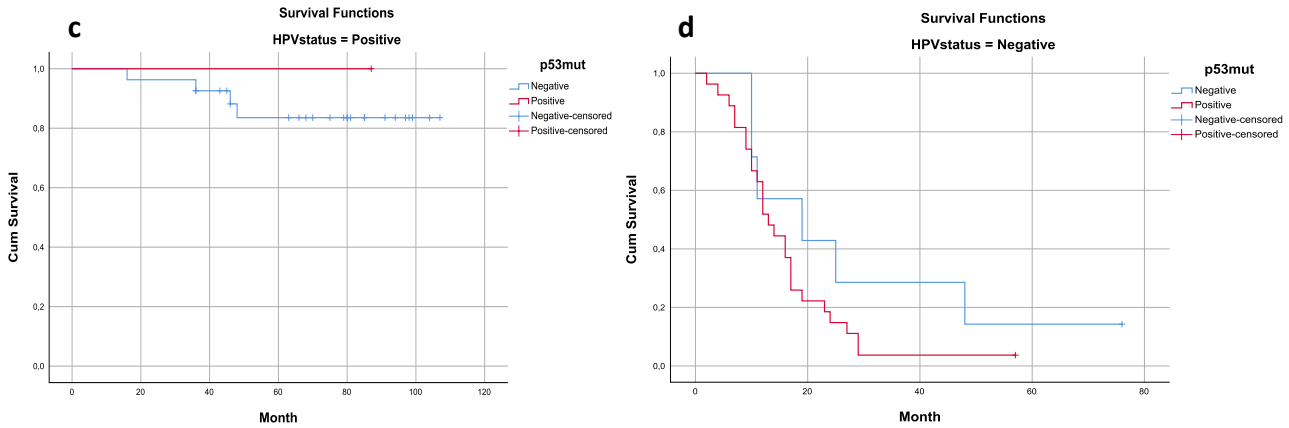


Figure 4: Overall survival of patients depending on HPV status for PD-L1 (a+b) and p53mutant (c+d) expression.

DISCUSSION

Determining the presence of HPV in OPSCC is very important for creating a molecular profile of the tumor and further treatment planning and should be included in the routine diagnosis, since transcriptionally active HPV determines this subtype of carcinoma. OPSCC occurs at a relatively young age, has a high sensitivity to treatment and a specific biological pattern of development. Data analysis states that simultaneous positivity HPV status and PD-L1 improve OS scores compared to their negativity (p=0.261). If we analyze by PD-L1 expression levels, then its low and medium levels reduce the risk of death in patients and they are often found in the HPV+ group, compared to high and very high levels increase the risk of death and most of all belong to the HPV− group (p=0.019). In all cases, a negative correlation was observed between the p53mutant protein and HPV status, which were assessed as counteracting factors (p<0.001). Only in the HPV− group did negative expression of p53mutant lead to a slight prolongation of patient's OS compared to positive expression (p<0.001). High and very high levels of p53mutant protein expression reduce the survival time of patients to a greater extent than low and medium levels. Although the p53wild protein is detected in a small number of patients, it demonstrates a positive correlation with HPV status as a strong factor that reduces the risk of death (p=0.01). In addition to these molecular predictors, other factors, such as age, smoking and alcohol use, were also analyzed, which are negatively correlated with HPV status and increase the risk of death (all p<0.05).

Persistent viral infection or a characteristic tumor microenvironment will trigger the depletion of cytotoxic T-cell action by upregulating the expression of a coinhibitory ligand to evade the immune system.[9] PD-L1 is a cell surface protein that can be expressed on a variety of tumor cells, macrophages, T cells, and other cells that plays a critical role in generating persistent HPV infection as well as resisting immune clearance during cancer development.[10] Many researchers evaluate PD-L1 protein expression only as positive and negative, or high and low with a cutting threshold value, or expressed as a division into several groups that differ sharply from each other. Considering that PD-L1 receptor expression has a dynamic characteristic, we calibrated a wide range of expression with different predictive value depending on the level of activity, taking into account HPV status, in order to obtain more comparable results. High levels of PD-L1 expression may indicate a high pathogenicity of tumor cells, an immunosuppressive microenvironment, or an intense T-cell attack armed with the PD-1 receptor. Thus, PD-L1 looks like an evasive molecular instrument, but features unknown to us determine its main function. The latest discovery that blocking the interaction of PD-L1 with PD-1 enhances the effectiveness of T-lymphocytes and the body's own immune system can recognize neoantigens and initiate an adaptive response against tumor cells, which has led to the strengthening of anticancer therapy and has become an exciting direction in the fight against carcinomas.

According to the authors, sequencing of human cancer genomes revealed that, in head and neck cancer, the mutation of the *TP53* gene is observed in 60% of cases.[11] Approximately 60-70% of *TP53* mutations are mostly missense in one allele with suppression of the second allele due to loss of heterozygosity (LOH). The remaining group (30-40%) did not undergo LOH, retaining the wild-type *TP53* allele.[12] In addition, many mutations in the *TP53* gene result in neoformal activation GOF (gain-of-function), which can enhance tumor progression, metastatic potential, and/or drug resistance when the p53mutant protein is overexpressed, even suppressing p53wild activities in cells (dominant-negative mechanism).[12,13] This is of great clinical importance because cells with positive p53wild expression without mutant-type activation are the most sensitive to chemoradiotherapy, which may be why HPV-induced tumors often exhibit higher radiosensitivity. Unlike to the most common missense mutations in the *TP53* gene, a deletion or nonsense mutation often results in the deletion or incomplete translation of the p53 protein due to residual transcriptional activity.[14] Thus, modulation of co-

translational folding during synthesis ensures the formation of an immature native structure of the p53 protein. This formation of molecularly incorrect polypeptide chains can lead to the loss of specific binding sites for targeted antibodies upon immunohistochemical staining. Based on the results of our study and the above data, it should be emphasized that the lack of expression of p53wild in the HPV− group can be explained by the suppression of the intact allele due to the LOH or an increase in the GOF of p53mutant. Despite the degradation of the p53 protein under the influence of the HPV E6 oncoprotein, the wild variant of the protein is found in a small amount in HPV+ OPSCC. The rare occurrence of mutations in the *TP53* gene in HPV+ OPSCC ensures the preservation of p53wild activity. More than half of the patients in the total group (54.8%) did not have a *TP53* gene mutation, it is possible that there may be deletions or nonsense mutations among them, because it is somewhat difficult to identify these structurally incorrect folding proteins immunohistochemically.

The longer survival of patients with HPV+ OPSCC compared with HPV− OPSCC has prompted the development of various strategies for de-intensification of treatment, in connection with which a lot of scientific research is being carried out in this direction on a global scale. However, HPV+ status is not always the only predictor of risk stratification for treatment reduction in OPSCC patients. Therefore, from our point of view, in order to reliably strengthen the resource of prognostic molecular predictors in addition to HPV status, it is necessary to identify additional indicators canonically related to the development of OPSCC. Based on our results, tumors that have well variation in the expression of molecular markers: «p16 70-100% (HPV status positive)/PD-L1 0-30% TPS/p53wild positive/53mutant negative» represent an ideal population for deintensification trials given their extremely low risk of death and excellent survival, as they are mostly HPV+ patients. Tumors that have poor variation of expression of molecular markers: «p16 0-70% (HPV status negative)/PD-L1 30-100% TPS/p53wild negative/p53 mutant positive» will show the opposite result, and mostly they are HPV− patients.

CONCLUSION

The convergence of molecular biology with clinical oncology has led to the development of revolutionary approaches with the right diagnosis and the use of innovative therapeutic products to help make the right decisions in complex clinical settings. According to the results of this analysis, HPV status and p53wild can be considered as protective factors, p53mutant, smoking, alcohol and age are risk factors, PD-L1 is located between them, with no apparent initiative. Thus, positive HPV and p53wild protein status are reliable protective factors, and the PD-L1 protein also belongs to this stratum only in a very weak amplitude and with low statistical significance. Other predictors are included in the risk group and adversely affect the survival of patients. After integrating the survival of patients with p53mutant, PD-L1, p53wild and other predictors, it was possible to study them as separate independent factors that can influence the outcome of OPSCC. Thus, the identification of reliable predictors is a prerequisite for improving the accuracy of prognosis and stratifying patients for individual treatment plans that do not have unnecessary toxic effects and are more effective. These findings should be interpreted with caution, as larger studies are needed to provide more accurate and spatial findings.

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