RESEARCH ARTICLE

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PI3K/AKT Immunoexpression Influences the Prognostic Factors of Patients Diagnosed with Oropharyngeal Squamous Cell Carcinoma

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Abstract

Background: Squamous cell carcinoma (SCC) is the most common histological type in the oropharynx with high incidence and mortality. The Pi3K-AKT signaling pathway is important for understanding prognostic factors such as metastasis, which is involved in the aetiopathogenesis of cancer and is associated with angiogenesis, progression, and cell survival. The aim of this study was to evaluate the correlation of the Pi3K/AKT pathway with prognostic factors associated with oropharyngeal SCC, including overall survival. **Materials and Methods:** Patients diagnosed and treated for oropharyngeal SCC at the Haroldo Juaçaba Hospital were included in the study. Clinicopathological and socio-demographic data were collected by reviewing patient records and reports. Excisional biopsies were analyzed by immunohistochemical techniques using TMA for the detection of antibodies against ANTI-AKT, ANTI-Pi3K, ANTI-p16, ANTI-Ki67, and ANTI-p53. Immunoexpression was evaluated qualitatively and quantitatively using ImageJ software, and data were correlated with patient survival. **Results:** There was a significant reduction in nuclear Pi3K immunoexpression in perilesional tissue compared to primary tumors and nodal metastases ($p<0.001$). Cytoplasmic Pi3K was increased in primary tumors and decreased in nodal metastases ($p=0.001$). pAKT expression in p16-negative tumors was associated with poorer overall survival. Further studies are needed to understand how this pathway may influence tumor progression and multimodal therapies. **Conclusion:** The *PI3K/AKT* pathway regulates cell survival and apoptosis and is associated with the malignant transformation of oropharyngeal tumors. AKT expression in p16-negative tumors is a strong predictor of poor prognosis.

Keywords: Survival- Squamous Cell Carcinoma of Head and Neck- Signal Transduction- *PI3K/AKT*- Oropharynx

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Introduction

Oropharyngeal cancer has a high incidence worldwide, with a particularly high prevalence in men over the age of 50 [1-6]. The primary sites of involvement are the soft palate, lingual tonsils, and the lateral and posterior walls of the oropharynx. The precise mechanism by which smoking and alcohol consumption act as primary predisposing factors remains unclear.

Nevertheless, the existing literature also indicates the potential impact of biological factors, such as exposure to the human papillomavirus (HPV), in the development of squamous cell carcinoma (SCC) in the oropharynx [7, 8]. Biological markers are of great importance in providing detailed information about tumor biology, including data on cancer status and progression. This is essential for determining the most appropriate therapeutic approach [9]. In the context of the oropharynx, the p16 protein is of particular significance as a prognostic marker, with positive tumors generally associated with a more favorable prognosis than negative tumors [8, 10].

In head and neck carcinomas, alterations in the signaling pathways that regulate the cell cycle and the DNA damage response are evident [11]. Among the signaling pathways frequently implicated in the etiology of squamous cell carcinoma (SCC), the PI3K-AKT pathway stands out, as it undergoes constant modifications in malignant neoplasms. These modifications correlate with processes such as metastasis, neovascularization, cell cycle progression, tumor growth, and the lesion's ability to resist the established treatment [11]. The activation of this pathway is initiated by the binding of growth factors to

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tyrosine kinase receptors on the cell membrane. Following this binding, the Pi3K molecule initiates a signaling cascade that, through its byproduct (PIP3), promotes the activation of AKT.

The phosphorylation of AKT generates by-products that result in the inactivation of the PTEN protein. The loss of PTEN results in an increase in AKT activity via the PI3K pathway, which ultimately leads to a reduction in apoptosis and the promotion of tumor cell survival. It is noteworthy that the PI3K oncogene, which is frequently amplified in oral and oropharyngeal cancers, is associated with more advanced disease stages, including invasion and metastasis. Additionally, there is a high incidence of mutations in this gene in cases where these advanced stages are present [12]. The activity of PI3K is stimulated by the Ras protein, and its action occurs via an indirect interaction. Specifically, the phosphatidylinositol 3-kinase (PI3K) enzyme phosphorylates phosphatidylinositol 4,5-bisphosphate (PtdIns-4,5-P2) to produce phosphatidylinositol 3,4,5-trisphosphate (PtdIns-3,4,5-P3). This process is dependent on the localization of the protein kinase B (AKT) on the plasma membrane, which is mediated by the phosphoinositide kinase. This kinase phosphorylates two residues in AKT: threonine 308, which is activated by PDK1, and serine 473, which is activated by mTOR kinase. This results in the full activation of AKT and a substantial increase in its activity [12, 13].

The protein kinase B (AKT) has been demonstrated to function as an effective inhibitor of apoptosis by phosphorylating and inactivating the BAD protein, which is a pro-apoptotic protein. This suggests that it plays a crucial role in regulating cell survival. The activity of the PI3K-AKT pathway has been widely associated with treatment failure in malignant lesions, such as squamous cell carcinoma. In a study comprising 53 patients undergoing multimodal therapy, a significant association was observed between AKT activation and treatment failure, thereby underscoring the relevance of this pathway in therapeutic resistance [13].

Additionally, evidence indicates a discrepancy between the sensitivity of head and neck carcinomas to radiotherapy and their resistance to the therapeutic modality when increased signaling through the Pi3K-AKT pathway is observed [12]. Nevertheless, the relationship between these proteins and the development of oropharyngeal cancer, as well as their role in predicting the prognosis of this disease, remains unclear and inconclusive. This underscores the necessity for further research in this field. The objective of this study is to analyze the immunoexpression of Pi3K/AKT in squamous cell carcinoma of the oropharynx, correlating it with prognostic factors and proliferation markers. The aim is to gain insight into the influence of these markers on the tumor microenvironment and to consider them as potential therapeutic targets in the treatment of oropharyngeal neoplasms.

Materials and Methods

Type of study and sample design

This study is a quantitative, observational, and cross-sectional study that has been approved by the Research Ethics Committee of the Haroldo Juaçaba Hospital/ Ceará Cancer Institute (HHJ/ICC) with opinion number 1.618.057.

The sample size was calculated based on the findings of Chaves. [11], who observed a higher expression of pAkt in oral squamous cell carcinomas compared to oral epithelial dysplasias (7.9 ± It was estimated that 50 cases would need to be evaluated to obtain a sample that represents, with 90% power and 95% confidence, the alternative hypothesis of this study (8.1 vs. 21.2±19.0%).

A total of 50 cases of patients who had undergone surgery for the resection of SCC in the oropharynx were selected for inclusion in the study. The exclusion criteria were any neoadjuvant therapy, incomplete records (>30% of socio-demographic and clinicopathological information), loss of follow-up, or insufficient material in the block and/or slide of the resected surgical specimens.

Collection of clinicopathological data

The socio-demographic data, including the patient's age, sex, family history of cancer, previous alcohol consumption, and smoking status, were extracted from the patient charts and histopathology reports and subsequently analyzed.

The dates of treatment commencement (day, month, and year) and the date of death (day, month, and year) or the conclusion of follow-up/discharge (for patients who did not progress to death) were obtained. The difference between these dates was calculated to determine the overall survival. These data were then subjected to statistical analysis to determine the number of months that elapsed between the aforementioned dates. Once the patients had been selected, the blocks were chosen and assembled for the creation of histological slides, which were then prepared for review and reclassification of the tumor gradation by an experienced pathologist.

Fabrication of Tissue Microarray Blocks and Immunohistochemistry Reaction

The blocks were selected, and from them, segments were collected representing an area of the excisional biopsy with adequate tumor parenchyma, representing the tumor front and satisfactorily representing the primary tumor (PT). Additionally, squamous epithelium from the surgical resection margin was included. (SRM); and a sample of metastasis in cervical lymph nodes (LNM) for patients who had this condition [14]. Following the selection of the slides, the respective blocks (donor block) were used to create the TMA block (recipient block).

To perform the TMA technique, a tissue microarray device (Quick-Ray UNITMA®) was utilized, puncturing a tissue fragment with a diameter of 2 mm that exhibited greater representativeness of the donor block. This fragment was then transferred in an orderly manner to the receptor block, which exhibited a precisely spaced matrix pattern.

The recipient block was submitted to sequential cuts of 4 μm thick, which were deposited on silanized glass slides for conventional hematoxylin-eosin staining and immunohistochemistry reactions.

Following deparaffinization and rehydration, antigen retrieval was performed by heating the tissue at 97°C for 20 minutes. The immunohistochemistry reaction was then conducted, after which the endogenous peroxidase was blocked with 3% hydrogen peroxide diluted in phosphate buffer solution (PBS). The slides were cooled to room temperature and washed with phosphate buffer solution (PBS). Subsequently, further washes in the buffer solution were performed, followed by an overnight incubation period with the anti-PI3K antibody (1:400, ab86714, Abcam®), the anti-pAkt antibody (1:100, EP2109Y, Abcam®), and the anti-Ki67 antibody. The following antibodies were used: DAKO® MIB1 (ready for use), DAKO® anti-p53 (ready for use), and DAKO® anti-p16 (ready for use). The latter was employed for pathological staging reclassification of tumors into p16+ and p16- [8].

Following an incubation period with primary antibodies, as specified by the manufacturer, the slides were washed with PBS and subsequently incubated with biotinylated polymer for 30 minutes. Following this, the slides were washed again and then conjugated with avidin-biotin peroxidase or the Envision® system for a further 30 minutes. The development was conducted using 3,3'-Diaminobenzidine (DAB) (DAKO®) and counterstaining with Harris hematoxylin at 7%.

Positive controls of the immunohistochemical reaction were performed using conventional histological sections, as recommended by the manufacturer. For the negative control, TMA slides were used, employing the same immunohistochemical technique described above, but omitting the incubation step with the primary antibody.

Immunohistochemical analysis

The immunohistochemical analysis was performed using five-field photographs taken at 400x magnification per histological section on a microscope with an attached camera (Leica DM 2000®) [15].

Quantitative evaluation was conducted using ImageJ® software, to which the photographs were exported. Cell counting was performed using the cell counter command, determining the percentage of labeled and unlabelled tumor cells, as well as the cellular location of this labeling, whether in cytoplasm, nucleus, or membrane. For the p16 analysis, samples were considered positive when strong and diffuse nuclear and cytoplasmic staining was detected in at least 75% of the tumor cells [3].

Statistical analysis

The data were tabulated in a Microsoft Excel spreadsheet and subsequently exported to the software Statistical Package for the Social Sciences (SPSS), where the analyses were performed with a confidence level of 95%.

Additionally, the means and standard deviation of the percentage of immunopositive tumor cells were calculated and correlated with the clinical characteristics through the use of statistical tests, including the Mann-Whitney and Kruskal-Wallis/Dunn. Subsequently, the mean and median overall survival time and 10-year survival rate were calculated based on the categorization of these values according to the median immunoexpression. Finally, these results were evaluated using Kaplan-Meier curves and the Log-rank Mantel-Cox test for comparison.

Results

Characterization of tumor microenvironment cell immunoexpression in OSCC

A total of 25 samples of perilesional tissue or surgical resection margin (SRM), 29 p16-primary tumor (PT) samples, 21 p16+ tumor samples, 16 p16-lymph node metastasis (LNM) samples, and 9 p16+ LNM samples were analyzed. A significant difference was observed in p53 immunoexpression, with a reduction in perilesional tissue when compared to other locations ($p < 0.001$). No statistically significant difference was noted in ki67 immunoexpression ($p = 0.344$) among the five groups (Figure 1).

The nuclear immunoexpression of P13K was found to be significantly reduced from perilesional tissue to primary and metastatic tumors ($p < 0.001$). No statistically significant differences were observed in the nuclear ($p = 0.176$) or cytoplasmic ($p = 0.253$) immunoexpression of pAkt between the groups. However, there was an increase in the cytoplasmic immunoexpression of Pi3K in the primary tumors and a decrease in expression in nodal metastases ($p = 0.001$) (Table 1).

The immunoexpression of these markers in primary tumors demonstrated no association with any of the clinical variables under investigation (Tables 2 and 3). However, p16-tumors in patients with a history of smoking exhibited higher immunoexpression for cytoplasmic pAkt. Patients with pAkt >30% exhibited a lower overall survival rate (p=0.035) (Table 4). In the multivariate analysis, nuclear (p=0.024) and cytoplasmic (p=0.014) immunoexpression for *pAKT* was directly associated with a worse prognosis (Table 5).

Discussion

The present study observed an increase in cytoplasmic Pi3K immunoexpression in primary tumors. While *pAKT* immunoexpression did not change significantly in the tissues studied, its expression was associated with a smoking history and lower overall survival in p16- tumors.

In p16-tumors, the primary risk factors are smoking and alcohol consumption. These factors are responsible for triggering mutations in several genes that are involved in the transcription of factors associated with cell cycle regulation and response to DNA damage. This leads to the activation of the *PI3K/AKT* pathway [11, 16]. It has been demonstrated that cigarette smoking is associated with increased *pAKT* expression in squamous cell carcinoma of the mouth and oropharynx.

This because nicotine, a constituent of cigarettes, affects signal transduction pathways that are of great relevance to the carcinogenesis of lesions in the lung, prostate, pancreas, mouth, and oropharynx [17]. It was

Table 1. *Pi3K/AKT* Pathway Immunostaining Characterization of SRM, PT and LNM.

	Nuclear (%)				Citoplasmático (%)	
Tissue	p53	ki67	PI3K	AKT	PI3K	AKT
SRM (n=25)	18.09±10.56	8.86±7.96	31.46±26.23*	1.00±3.57	22.83±29.94	40.71±36.20
PT p16- (n=29)	38.83±32.09	16.72±14.82	0.00±0.00	1.43±3.47	97.43±10.07*	36.20±29.71
PT p16+ (n=21)	36.23±31.95	11.49±14.57	0.00±0.00	0.30±0.63	100.00±0.00*	21.66±27.09
LNM p16- (n=16)	47.19±29.43	16.10±3.20	0.00±0.00	0.00±0.00	67.26±44.83*†	43.76±42.01
LNM p16+ (n=9)	31.88±28.84	22.42±15.07	0.00±0.00	0.00±0.00	72.04±45.36*†	40.83±35.45
p-Value[a]	0.913	0.344	<0.001	0.176	0.001	0.253

[a]Kruskal-Wallis/Dunn test, *p<0.05 versus other groups; †p<0.05 versus other groups; (mean ±SD). SRM, Surgical resection margins; PT, Primary tumors; LNM, Lymph node metastasis.

observed in this study that in p16-tumors, patients with a history of smoking exhibited higher immunoexpression for cytoplasmic *pAKT*, thereby reinforcing the role of smoking in the activation of this pathway.

The extant literature suggests that the pathways most commonly affected by nicotine include the pathway of activated protein kinase (MAPK), suppression of the apoptotic pathway mediated by cytochrome-c, and the activation of phosphatidylinositol-3-kinase (*PI3K/AKT*). The study by West, [18] provides evidence that nicotine can activate *pAKT*, resulting in cell survival and an anti-apoptosis response. These processes contribute to tumor progression, reinforcing the influence of the *PI3K/AKT* pathway.

Table 2. Cohort Clinicopathological and Risk Factor Features and Association with *PI3K/AKT* Pathway in OPSCC p16-.

	Niclear (%)				Citoplasmático (%)	
	p53	*ki67*	*PI3K*	*AKT*	*PI3K*	*AKT*
Sex						
Male (n=24)	41.86±33.21	16.77±15.20	0.00±0.00	1.31±3.63	96.50±11.69	38.44±31.37
Female (n=5)	27.31±27.35	16.46±15.32	0.00±0.00	1.76±3.19	100.00±0.00	29.75±25.03
p-Value[b]	0.749	0.762	1	0.411	0.386	0.465
Age						
≤60 (n=5)	32.34±25.56	30.22±19.49	0.00±0.00	1.40±3.43	100.00±0.00	36.62±32.61
>60 (n=24)	40.13±33.65	15.30±14.17	0.00±0.00	1.44±3.55	96.79±11.21	36.10±29.70
p-Value[b]	0.938	0.186	1	0.649	0.472	0.919
Family history of cancer						
Yes (n=5)	60.29±27.71	14.41±14.78	0.00±0.00	1.07±1.86	95.80±11.11	29.86±30.68
No (n=24)	33.18±31.36	17.65±15.26	0.00±0.00	1.53±3.84	97.93±9.95	38.05±29.84
p-Value[a]	0.07	0.845	1	0.357	0.395	0.599
Smoking history						
Yes (n=6)	28.15±30.60	19.98±16.44	0.00±0.00	3.47±5.41	94.70±15.90	32.74±31.18
No (n=23)	42.39±32.62	15.09±14.31	0.00±0.00	0.60±1.88	98.60±6.42	37.61±29.73
p-Value[a]	0.23	0.477	1	0.018	0.496	0.676
Alcohol consumption						
Yes (n=5)	36.02±34.23	21.47±15.89	0.00±0.00	3.64±6.11	92.05±19.47	37.77±31.54
No (n=24)	39.99±32.18	15.24±14.69	0.00±0.00	0.78±2.01	98.78±6.00	35.74±29.85
p-Value[a]	0.505	0.362	1	0.197	0.255	0.867
cT						
T1/2 (n=12)	35.94±34.40	9.46±11.64	0.00±0.00	1.58±3.73	96.03±13.77	35.69±30.99
T3/4 (n=17)	39.59±31.96	18.62±15.88	0.00±0.00	0.65±1.38	98.16±7.35	37.65±29.86
p-Value[a]	0.538	0.203	1	0.637	0.795	0.806
cN						
N0 (n=8)	40.32±41.72	6.06±7.55	0.00±0.00	0.00±0.00	100.00±0.00	43.09±31.18
N+ (n=21)	33.57±26.23	16.09±16.53	0.00±0.00	1.06±2.43	97.90±7.86	25.87±25.62
p-Value[a]	0.782	0.266	1	0.082	0.398	0.15

*p<0.05 versus other groups; [a]Mann-Whitney test; [b]Wilcoxon test; p<0.05 (mean ±SD). MSI, microsatellite instability.

Table 3. Cohort Clinicopathological and Risk Factor Features and Association with *PI3K/AKT* Pathway in OPSCC p16+.

	Nuclear (%)				Citoplasmático (%)	
	p53	*ki67*	*PI3K*	*AKT*	*PI3K*	*AKT*
Sex						
Male (n=14)	33.76±29.25	14.77±15.90	0.00±0.00	0.39±0.73	100.00±0.00	27.14±30.09
Female (n=7)	41.99±39.97	4.94±9.69	0.00±0.00	0.14±0.38	100.00±0.00	10.70±16.55
p-Value[b]	0.564	0.095	1	0.454	1	0.236
Age						
≤60 (n=7)	21.86±18.85	21.38±20.54	0.00±0.00	0.29±0.49	100.00±0.00	16.64±20.43
>60 (n=14)	42.38±34.92	7.90±10.83	0.00±0.00	0.31±0.71	100.00±0.00	24.17±30.26
p-Value[b]	0.364	0.263	1	0.803	1	0.782
Family history of cancer						
Yes (n=7)	33.46±32.17	13.55±20.63	0.00±0.00	0.10±0.26	100.00±0.00	13.30±20.61
No (n=14)	37.41±33.00	10.46±11.72	0.00±0.00	0.41±0.74	100.00±0.00	25.84±29.60
p-Value[a]	0.805	0.711	1	0.369	1	0.477
Smoking history						
Yes (n=4)	19.45±10.89	16.31±28.25	0.00±0.00	0.25±0.50	100.00±0.00	7.23±14.45
No (n=17)	40.42±34.29	10.29±10.82	0.00±0.00	0.32±0.67	100.00±0.00	25.06±28.52
p-Value[a]	0.45	0.56	1	1	1	0.169
Alcohol consumption						
Yes (n=8)	34.61±27.03	17.56±20.36	0.00±0.00	0.41±0.58	100.00±0.00	22.85±28.38
No (n=13)	37.30±35.99	7.45±8.15	0.00±0.00	0.24±0.68	100.00±0.00	20.93±27.41
p-Value[a]	0.877	0.552	1	0.265	1	0.878
cT						
T1/2 (n=9)	25.36±29.74	7.29±9.74	0.00±0.00	0.46±0.82	100.00±0.00	22.16±29.02
T3/4 (n=12)	40.74±32.63	14.29±17.03	0.00±0.00	0.21±0.47	100.00±0.00	23.23±27.27
p-Value[a]	0.409	0.44	1	0.484	1	0.905
cN						
N0 (n=6)	60.78±35.43	6.48±6.91	0.00±0.00	0.00±0.00	100.00±0.00	24.28±36.44
N+ (n=15)	23.85±25.22	11.14±15.52	0.00±0.00	0.46±0.73	100.00±0.00	18.43±21.15
p-Value[a]	0.111	0.886	1	0.135	1	0.922

*p<0.05 versus other groups; [a]Mann-Whitney test; [b]Wilcoxon test; p<0.05 (mean ±SD); MSI, microsatellite instability

Although HPV infection represents a significant risk factor for the development and evolution of cancer, positivity for p16 is associated with a more favorable prognosis, whereas negativity for p16 is associated with a poorer prognosis [19]. In this study, we observed a high expression of Pi3K in both p16- and p16+ tumors, with no significant differences in the immunoexpression of pAkt in the tumor region, regardless of whether p16- or p16+ status was present. The PI3K protein is expressed and undergoes constant changes in malignant neoplasms [11], establishing a relationship with processes such as metastasis, vascular neoformation, cell cycle progression, growth, and the ability of the lesion to resist implemented treatment [20].

It is frequently observed that mutations, as well as deregulations of signal relays in this pathway, are present in neoplastic tissue [16]. Pizón, [21] posits that as PI3K undergoes phosphorylation in the cytoplasm, leading to an increase in its activity, the subsequent phosphorylation of PIP2 will be converted into PIP3, thereby activating AKT. This allows us to observe a reduction in Pi3K following its phosphorylation and a subsequent increase in AKT due to its activation through Pi3K. These findings are in agreement with the results obtained in this study, which demonstrated that Pi3K was increased in the cytoplasm, particularly in primary tumors and nodal metastases, compared to perilesional tissue. This suggests an association with the malignant process of the lesion.

Nevertheless, some studies indicate that PIP3, a component of PI3K, is directed to the nuclear matrix and other nuclear sites, such as the inner nuclear leaflet and protein complexes, for anchoring or activation, through signal-dependent translocation of the cytoplasmic compartment [21]. This finding lends further support to the present study, which also observed the presence of Pi3K in the nucleus, presumably in an inactive form, at the margins of surgical resection. This suggests the presence of recycled Pi3K in the nucleus.

The AKT pathway is capable of inhibiting apoptosis via multiple routes, including the cytoplasmic action of BAD and the nuclear translocation of NFKB, which facilitates the transcription of anti-apoptotic genes.

Table 4. Cohort Clinicopathological and Risk Factor Features and Association with *PI3K/AKT* Pathway in p16- and p16+ OPSCC.

	p16-				p16+			
	10-years OS	(CI95%)	Median	p-Value	10-years OS	(CI95%)	Median	p-Value
All sample	18 (56.3%)	(41.00-74.05)	62	-	12 (57.1%)	(54.08-97.76)	-	-
Sex								
Male	12 (50.0%)	(29.67-63.06)	45	0.225	7 (50.0%)	(40.31-91.73)	33	0.325
Female	6 (75.0%)	(44.60-106.54)	.		5 (71.4%)	(58.44-125.56)	.	
Age								
≤60	6 (85.7%)	(61.03-103.54)	.	0.151	5 (71.4%)	(59.58-124.99)	.	0.308
>60	12 (48.0%)	(33.33-69.29)	45		7 (50.0%)	(35.60-79.97)	40	
Family history of cancer								
Yes	5 (62.5%)	(25.45-68.55)	62	0.89	4 (57.1%)	(42.62-87.66)	.	0.71
No	13 (54.2%)	(38.47-76.05)	45		8 (57.1%)	(46.84-102.16)	.	
Smoking history								
Yes	4 (44.4%)	(12.94-70.33)	21	0.299	2 (50.0%)	(17.99-97.51)	33	0.757
No	14 (60.9%)	(38.74-78.89)	62		10 (58.8%)	(53.57-101.75)	.	
Alcohol consumption								
Yes	3 (42.9%)	(11.52-56.19)	21	0.387	4 (50.0%)	(26.70-84.05)	33	0.495
No	15 (60.0%)	(40.89-78.26)	62		8 (61.5%)	(55.31-108.38)	.	
cT								
T1/2	7 (53.8%)	(30.73-75.48)	45	0.769	5 (55.6%)	(40.60-108.29)	.	0.729
T3/4	11 (64.7%)	(43.57-88.95)	.		7 (63.6%)	(51.27-107.15)	.	
cN								
N0	5 (50.0%)	53.82±14.18 (26.02-81.62)	45	0.477	4 (80.0%)	(46.95-103.05)	.	0.43
N+	11 (68.8%)	63.58±10.52 (42.95-84.20)	.		8 (57.1%)	(48.58-102.48)	.	
P53								
Até 20%	7 (70.0%)	72.67±13.87 (45.49-99.84)	.	0.199	6 (66.7%)	(49.37-117.30)	.	0.564
>20%	6 (42.9%)	30.91±5.89 (19.36-42.46)	26		5 (45.5%)	(34.08-78.40)	40	
ki 67								
Até 10%	2 (25.0%)	25.75±5.99 (14.01-37.49)	21	0.415	5 (55.6%)	(35.60-102.62)	.	0.64
>10%	7 (53.8%)	53.34±11.85 (30.10-76.57)	62		4 (66.7%)	(46.94-123.81)	.	
AKT nuclear								
0%	12 (52.2%)	57.55±9.29 (39.35-75.75)	62	0.703	10 (62.5%)	(59.40-106.60)	.	0.183
>0%	5 (62.5%)	41.98±11.57 (19.31-64.65)	.		2 (40.0%)	(10.38-82.82)	19	
AKT citoplasmático								
Até 30%	9 (69.2%)	75.17±10.89 (53.83-96.51)	.	0.035	8 (57.1%)	(50.77-103.23)	.	0.866
>30%	8 (44.4%)	34.71±8.12 (18.80-50.61)	24		4 (57.1%)	(27.41-70.59)	.	
PI3K citoplasmático								
<100%	1 (50.0%)	11.00±7.07 (0.00-24.86)	1	0.422	-	-	-	-
100%	15 (53.6%)	56.28±8.75 (39.12-73.44)	62		11 (55.0%)	(51.09-96.15)	.	

*p<0.05. Log-Rank Mantel Cox test; SE, standart error; IC95%, intervalo de confiança 95% da média de sobrevida; OS, overall survival.

This finding is consistent with the observations made by Chaves, [11], who analyzed oral SCC samples and noted that *pAKT* expression was increased in both the cytoplasm and nucleus, with no statistically significant difference between the two. This suggests that AKT may act independently in hyperproduction, as a result of its presence in the cytoplasm, and hyperfunction, as a result of its presence in the nucleus, through nuclear export [22, 23].

The present study revealed a notable elevation in p53 levels in tumors and nodal metastasis (p16+) and (p16-) when compared to perilesional tissue. Given that nuclear *pAKT* has decreased in these same locations, it is pertinent to highlight the findings of the study by Pizón, [21] regarding the effects of AKT on cell survival. AKT inactivates p53 through the action of MDM2, a protein that, when activated, negatively regulates p53 in the nucleus by binding to it and preventing its activity. Furthermore, in the cytoplasm, p53 levels are reduced through ubiquitylation and proteasomal degradation.

In vitro studies analyzing gastric, colorectal, and urothelial tumors have observed changes in the behavior of the *PI3K/AKT* pathway, particularly about factors

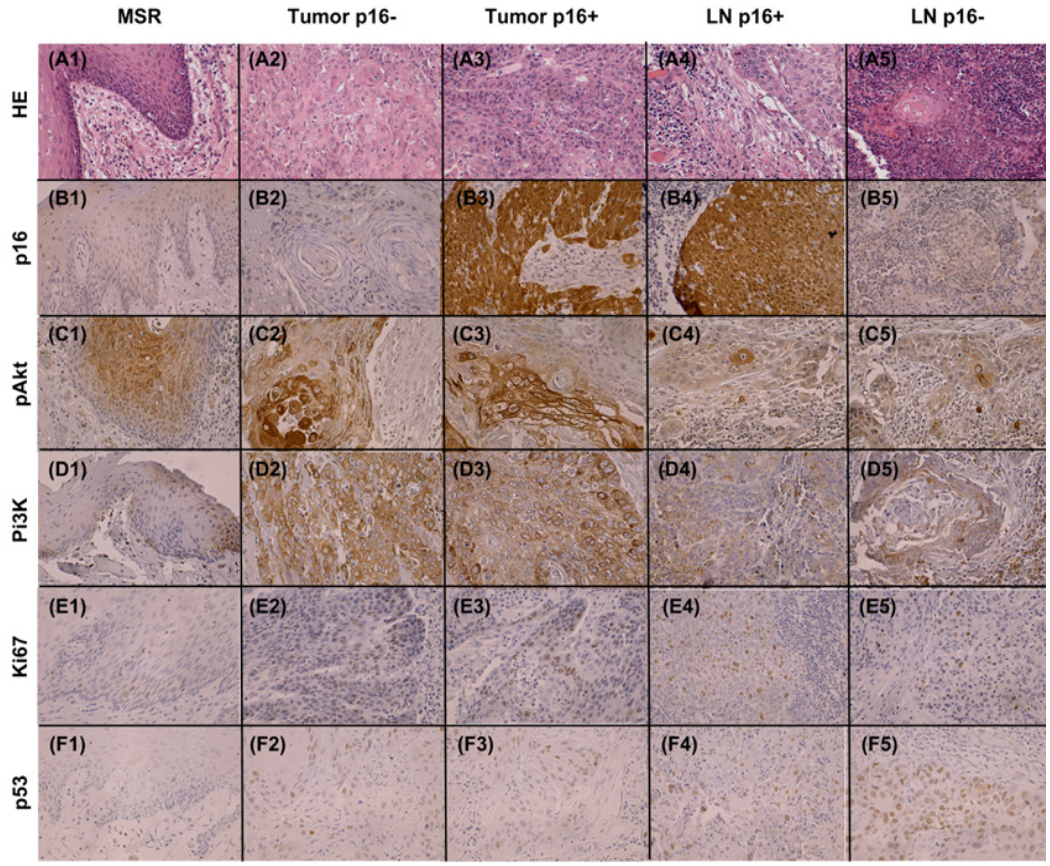

Figure 1. Immunoexpression of *Pi3K, pAKT, p16, Ki67* and *p53* in Surgical Resection Margin, Primary Tumor p16- and p16+, and Lymph node Metastasis p16+ and p16-. (A1) Surgical Resection Margin in HE; (A2) p16+ Tumor in HE; (A3) p16- Tumor in HE; (A4) p16+ Lymph node metastasis in HE; (A5) p16- Lymph node metastasis in HE. (B1) Immunolabeling for p16 in Surgical Resection Margin; (B2) Immunolabeling for p16 in p16+ Tumor; (B3) Immunolabeling for p16 in p16- Tumor; (B4) Immunolabeling for p16 in p16+ Lymph node metastasis; (B5) Immunolabeling for p16 in p16- Lymph node metastasis. (C1) Immunolabeling for pAkt in Surgical Resection Margin; (C2) Immunolabeling for pAkt in Tumor p16+; (C3) Immunolabeling for pAkt in Tumor p16-; (C4) Immunolabeling for pAkt in Lymph node Metastasis p16+; (C5) Immunolabeling for pAkt in Lymph node Metastasis p16-. (D1) Immunolabeling for Pi3k in Surgical Resection Margin; (D2) Immunolabeling for Pi3k in p16+ Tumor; (D3) Immunolabeling for Pi3k in p16- Tumor; (D4) Immunolabeling for Pi3k in p16+ Lymph node Metastasis; (D5) Immunolabeling for Pi3k in p16- Lymph node Metastasis. (E1) Immunolabeling for Ki67 in Surgical Resection Margin; (E2) Immunolabeling for Ki67 in p16+ Tumor; (E3) Immunolabeling for Ki67 in p16- Tumor; (E4) Immunolabeling for Ki67 in p16+ Lymph node Metastasis; (E5) Immunolabeling for Ki67 in p16- Lymph node Metastasis. (F1) Immunolabeling for p53 in Surgical Resection Margin; (F2) Immunolabeling for p53 in Tumor p16+; (F3) Immunolabeling for p53 in Tumor p16-; (F4) Immunolabeling for p53 in Lymph node metastasis p16+; (F5) Immunolabeling for p53 in Lymph node metastasis p16-

such as smoking and alcohol consumption. The same was observed through the analysis of the expression of Pi3k in different tumor locations, including the colon, ovary, cervix, gastric, and lung [24, 25]. Additionally, studies have indicated that the *PI3K/AKT*/mTOR pathway may contribute to resistance to chemotherapy and

Table 5. Predictors of Overall Survival of Patients with OPSCC p16- and p16+ according to Multinomial Logistic Regression Model.

	p16-				p16+			
	p-Value	HR	CI95%		p-Value	HR	CI95%	
Overall survival								
ki67 nuclear	0.095	0.95	0.9	1.01	0.189	1.04	0.98	1.11
p53 nuclear	0.199	0.98	0.95	1.01	0.172	1.02	0.99	1.06
AKT nuclear	*0.024	1.25	1.03	1.52	0.051	5.43	0.98	29.54
AKT citoplasmatico	*0.014	1.04	1.01	1.08	0.37	0.98	0.95	1.02
p13K nuclear	-	-	-	-	-	-	-	-
p13K citoplasmatico	0.48	0.98	0.91	1.04	-	-	-	-

*p<0.05. Cox regression. HR, hazard risk for dead; CI, confidence interval. HR and CI estimated by Cox proportional hazard regression model.

radiotherapy, which could result in a poorer prognosis and, subsequently, reduced overall survival [26-28]. These findings align with those observed in the current study, where *pAKT* immunostaining was associated with poorer overall survival in p16-tumors.

This study is limited by its retrospective design and the inability to fully elucidate the mechanism by which the pathway acts in tumor progression despite identifying the locations of immunoexpression of the evaluated proteins. However, this study demonstrates the behavior of this signaling pathway in preneoplastic and neoplastic tissues of oropharyngeal SCC.

In conclusion, the *PI3K/AKT* pathway plays a pivotal role in regulating tumor cell survival and apoptosis. The presence of PI3K is associated with the malignant transformation of oropharyngeal tumors, whereas *pAKT* expression in p16- oropharyngeal tumors has been observed as a robust predictor of poor prognosis. However, further studies are required to elucidate the mechanisms through which this pathway may interfere with both tumor progression and multimodal therapies and radiotherapy.

Author Contribution Statement

All authors contributed to the conception and design of the study. Jennifer Vianna Barbosa, André Alves Crispim, and Jeferson Virgulino Sousa were responsible for data collection and manuscript writing. Paulo Goberlânio de Barros Silva, Thinali Sousa Dantas, and Jennifer Vianna Barbosa were responsible for data analysis and interpretation. Thinali Sousa Dantas, Fabrício Bitu Sousa, and Paulo Goberlânio de Barros Silva developed the concept and design of the study, critically revised the manuscript for important intellectual content, reviewed the text and manuscript writing, and approved the final version. Karuza Maria Alves Pereira and Maria do Perpétuo Socorro Saldanha Cunha participated in text review and manuscript writing and approved the final version. All authors read and approved the final manuscript.

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Ethical Issues

The work was approved by the Research Ethics Committee of the Haroldo Juaçaba Hospital / Ceará Cancer Institute (HHJ/ICC) with opinion number 1.618.057.

Conflict of interest

There is no conflict of interest.

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