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ORIGINAL ARTICLE

Triple Positive Oral Squamous Cell Carcinoma Patients Predict Poor Survival Outcomes: Multiple Factor Positivity Warrants the Need for Modified Treatment Approaches

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Abstract

Objectives The presence of lymphovascular invasion (LVI), perineural invasion (PNI) and extranodal extension (ENE) have shown adverse outcomes in oral squamous cell carcinoma (OSCC). This study evaluated the impact of LVI, PNI and ENE, individually and in combination, on survival outcomes in OSCC.

Material and Methods A retrospective analysis of a prospectively maintained oral cancer database was done from January 2017 to March 2023. All consecutive OSCC patients who underwent curative intent surgery were included. The triple-positive group was defined by the presence of all three features (LVI/PNI/ENE), while the double-positive group had the presence of two features. The disease-free survival (DFS) and overall survival (OS) analysis was done between different study groups.

Results A total of 255 patients were included in the analysis. The LVI, PNI and ENE positivity was 13%, 26% and 11%, respectively. There were 19 patients (7%) with double-positive and ten patients (4%) with triple-positive disease. The triple-positive group had lower DFS than non-triple-positive (0% vs 57%, *p*-value 0.001) and lower OS (0% vs 72%, *p*-value 0.003). The median DFS and OS of the triple-positive group were eight months and 24 months, respectively. Similarly, the double-positive group also had statistically significant inferior DFS (*p*-value 0.007) and OS (*p*-value 0.002) compared to the single-positive/triple-negative group.

Conclusion The triple-positive disease had poor outcomes, with no patients achieving disease-free or overall survival at the 5-year follow-up. The presence of multiple adverse factors necessitates modification of adjuvant therapy and therapeutic strategy, which may enhance survival outcomes.

Keywords Prognosis · Oral cancer · Head and neck cancer · Survival analysis · Disease-free survival · Lymphovascular invasion · Perineural invasion · Extranodal extension

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Introduction

Despite advancements in cancer management, the overall prognosis of oral squamous cell carcinoma (OSCC) has seen no significant improvement over the past few decades [1]. The biology of OSCC has many uncertainties;

hence, researchers have extensively tried studying various clinico-pathological factors to predict survival outcomes and tailor adjuvant treatment. Apart from standard TNM staging, several other microscopic features predict the severity and prognosis of the disease. Previous meta-analysis have shown that tumour thickness, depth of invasion (DOI), lymphovascular invasion (LVI), perineural invasion (PNI), extranodal extension (ENE) and status of surgical margins are independent prognostic markers in OSCC [2]. Frequently patients will have more than one of these factors. However, the data on the combination of such factors and their impact on the survival outcomes in OSCC is sparsely studied.

Although previous studies have identified LVI and PNI as independent prognostic markers in OSCC, their role as definitive indications for adjuvant therapy (radiotherapy or chemoradiotherapy) is not well established due to contrasting results [3–8]. In a recent study by Huang et al., which analysed 127 patients with tongue SCC, the double-positive disease (LVI + /PNI +) was found in 5% of cases. It was associated with significantly poorer overall survival, with a median survival of 10 months [9]. Another study by Ting et al. included 98 OSCC patients, of which 27 (27.6%) had the double-positive disease (LVI + /PNI +). The presence of the double-positive disease correlated with higher lymph node and distant metastasis rates with poorer 5-year disease-specific survival [10]. The AJCC 8th edition staging system has incorporated ENE based on evidence from a multicenter study demonstrating its impact on disease-free survival [11, 12]. The presence of ENE is an indication of adjuvant chemoradiotherapy based on the long-term follow-up result of a randomised controlled trial [13]. However, the outcomes associated with combining ENE with other adverse factors have not yet been extensively studied.

Triple-positive OSCC is characterised by the simultaneous presence of three adverse histopathological features: LVI, PNI and ENE. There has been only one previous study on triple-positive OSCC conducted by Lin et al., which included a cohort of 554 patients. In their study, the rate of triple positivity was found to be 2.7% ($n = 15$), and these patients exhibited a dismal 5-year disease-free survival (DFS) and overall survival (OS) of 27% and 20%, respectively. Notably, Lin et al. also explored the combined impact of two out of three adverse features. They concluded that even two adverse features were associated with lower OS rates [14]. Apart from this study, the data on triple-positive OSCC is scarce and its management strategy is not well discussed. Hence, we conducted this study to evaluate the impact of LVI, PNI and ENE individually and in combination on survival outcomes in OSCC with a future guide towards treatment options in this cohort of patients.

Material and Methods

Study Design, Setting and Participants

A retrospective analysis of a prospectively maintained oral cancer database was done from January 2017 to March 2023 at an academic tertiary care centre in Eastern India. The last follow date was 1st June 2023. All consecutive oral squamous cell carcinoma patients who underwent curative intent surgery were included in the study. Exclusion criteria comprised patients with recurrent disease, those who did not undergo neck dissection, and individuals with missing data on LVI, PNI, and ENE. Institutional Ethics Committee (IEC) approval was taken before the commencement of the study. The study adhered to the guidelines outlined by the Declaration of Helsinki, the International Council for Harmonisation (ICH)—Good Clinical Practice (GCP), and the Indian Council of Medical Research (ICMR).

Workup and Treatment Protocol

All patients underwent comprehensive history taking and thorough clinical examinations at the initial presentation. A punch or edge biopsy was performed to establish the histopathological diagnosis. Radiological imaging, such as contrast-enhanced computed tomography (CECT) or magnetic resonance imaging (MRI), was conducted based on the specific subsite involved. The 8th edition of the AJCC TNM staging system was utilised to classify the disease stage [15]. The patients with borderline extensive locally advanced disease underwent neoadjuvant chemotherapy (NACT); the rest underwent upfront surgery. The surgery performed was a wide local excision of the primary lesion and neck dissection with or without reconstruction. The resected specimen was sent for histopathological examination, and reporting was done based on the CAP guidelines [16].

The decision regarding adjuvant therapy was made through a multidisciplinary tumour board meeting. Patients with T3, T4, closer margin/s or N1 disease received adjuvant radiotherapy. Those with extranodal extension (ENE) or positive surgical margins underwent adjuvant cisplatin-based chemoradiotherapy. Patients with other risk factors, such as depth of invasion (DOI), lymphovascular invasion (LVI), or perineural invasion (PNI), received adjuvant radiotherapy or chemoradiotherapy on a case-by-case basis. Post-operative follow-up was recommended for all patients, with visits scheduled every three months for the first two years, every six months from the second to fifth year, and annually thereafter. At each visit, any oncological events, including recurrence or mortality, were recorded in the departmental database and documented.

Study Variables

Clinical and demographic parameters, such as age, sex, comorbidities, and treatment details, including neoadjuvant therapy, surgical findings, and adjuvant therapy, were retrieved from the database. The pathological data like the stage of the disease, status of surgical margins, grade/differentiation, lymph node status, LVI, PNI and ENE were also analysed, on surgical resected specimens. The principal objective of our study was to assess the impact of a concurrent presence of three adverse histopathological prognostic markers—LVI, PNI and ENE. Recognizing that the prognostic significance of established factors such as disease stage and grade is widely acknowledged, the focus was to study the implications of co-occurrence presence of LVI, PNI, and ENE positivity, termed as "triple positive oral cancer" on the survival outcomes. A strict criteria and standard guidelines / protocols were used to define LVI, PNI and ENE. The LVI was defined as the presence of tumour cells within a definite endothelial-lined space (lymphatics or blood vessels) in the tissue surrounding invasive carcinoma [3]. The segregation between invasion of lymphatics or blood vessels was not attempted. PNI was defined when the tumour was close to the nerve and at least involved one-third of its circumference or tumour cells within any three layers of the nerve sheath. Mere entrapment of nerve in tumour was not considered as PNI [17]. ENE was defined as an extension of metastatic tumour present within the confines of the lymph node, through the lymph node capsule into the surrounding connective tissue, with or without an associated stromal reaction [16]. The extent of pathological ENE as macroscopic (> 2 mm) and microscopic (≤ 2 mm) was not determined. All the cases included in the study were reviewed and reported by two pathologists. Histopathological images of the study variables are shown in Fig. 1.

Study Groups

The patients were categorised into groups based on the presence or absence of three pathological parameters: LVI, PNI and ENE. Patients with all three negatives were labelled as Group A (Triple Negative). The patients with the presence of either one of LVI, PNI or ENE were designed as Group B (Single Positive) and with two parameters positive were described as Group C (Double Positive). The patients in Group D (Triple Positive) were defined by the presence of all three pathological parameters. A comparative analysis was performed, comparing Group D (Triple Positive) with the combined group of Group A, Group B, and Group C (Non-Triple Positive). A separate analysis was conducted, comparing Group C (Double Positive) with the combined group of Group A and Group B (Single Positive and Triple Negative).

Statistical Analysis

The data was retrieved from the database in an Excel sheet. The continuous data were described as mean ± standard deviation (SD). The category variables were described using percentages (%). The student t-test was used to analyse continuous data, and the chi-square or Fischer's exact test for categorical variables. The survival outcomes were studied using the Kaplan–Meier method. The Cox regression hazard model was used to calculate the hazard ratio (HR), 95% confidence interval (95%CI) and correlation of variables with survival outcomes. The univariate and multivariate analyses were conducted on potential adverse clinical-pathological features to assess their association with survival outcomes. Disease-Free Survival (DFS) was defined as the duration from the surgery date to the occurrence of recurrence or death or until the last follow-up date. Overall Survival (OS) was defined as the duration from the date of diagnosis to

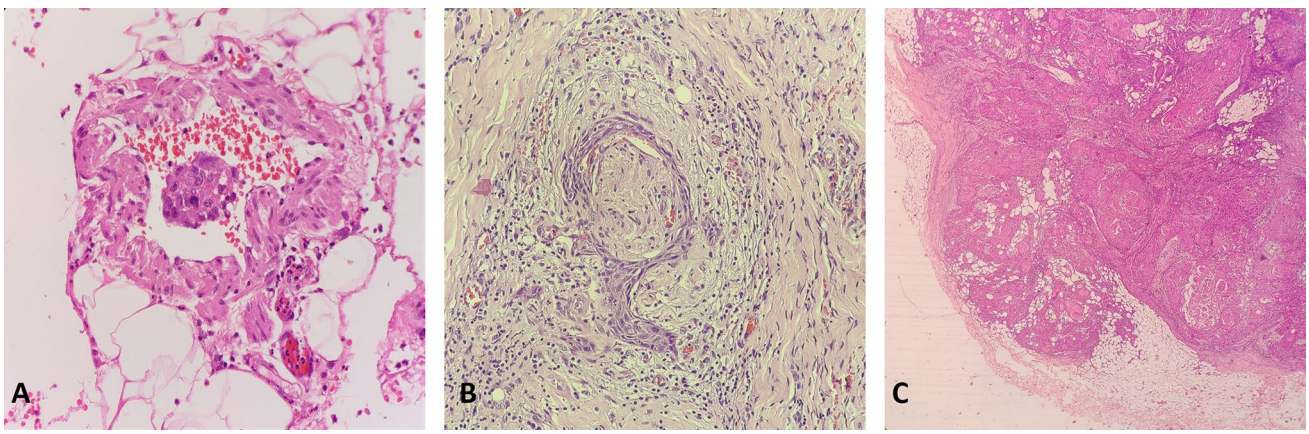

Fig. 1 Histopathology images of the study varaibles. **A** Lymphovascular invasion (LVI), H&E Stain 400x. **B** Perineural Invasion (PNI), H&E Stain 400x. **C** Extranodal Extension (ENE), H&E Stain 400x

death from any cause or until the last follow-up date. Statistical significance was defined as a p-value ≤ 0.05. All statistical analyses were performed using Stata 15.0 software (STATA Corp, College Station, TX, USA).

Results

Population Characteristics

A total of 280 patients underwent curative intent surgery for oral squamous cell carcinoma in the department during the study period. After excluding the patients who did not undergo neck dissection ($n=10$), with recurrent disease ($n=4$) and with missing data ($n=11$), 255 patients were included in the final analysis. The mean age of the cohort was 52 years, with the majority being male patients (209, 82%). The most commonly affected subsite was the gingivo-buccal complex (162, 64%), followed by the tongue (88, 34%). Neoadjuvant therapy was administered to 38 patients (15%). All 255 patients underwent wide local excision of primary lesion and neck dissection with or without reconstruction. The final histopathological surgical margin was involved by carcinoma in 9 patients (4%), while the rest 246 patients (96%) had close or clear surgical margins. The most common tumour stage was T4 (82, 32%), followed by T2 (74, 29%), T1 (50,20%) and T3 (49,19%). Lymph node involvement was present in 98 patients (38%). Regarding tumour grade, grade 1 was the most prevalent (177, 69%), followed by grade 2 (67, 26%) and grade 3 (12, 5%). Lymphovascular invasion (LVI) was found in 32 patients (13%), perineural invasion (PNI) in 68 patients (26%), and extranodal extension (ENE) in 29 patients (11%). Adjuvant radiotherapy was administered to 105 patients (41%), while 62 (24%) received adjuvant chemoradiotherapy. The detailed clinicopathological parameters of the study population are mentioned in Table 1.

Analysis of DFS and OS

The median follow-up of the study was 24 months (range, 2–95 months). A total of 211 patients (83%) were operated one year prior to the last follow up date. There were 61 patients with recurrence (24%), and 55 patients had died (22%). The 5-year DFS of the entire cohort was 53% (Fig. 2A). On univariate analysis, advanced TNM stage (III/IV), grade 3 disease, LVI, PNI and ENE showed poorer DFS. (Table 2) However, on multivariate analysis, only the advanced TNM stage showed a statically significant correlation with DFS. The univariate and multivariate analysis of DFS is described in Table 3

The 5-year overall survival (OS) rate for the study population was 68% (Fig. 3A). Univariate analysis

Table 1 Clinico-pathological parameters of the study population

Clinicopathological parameters	Number of patients ($n=255$)	Percentage
Age		
(Mean + SD)	52 ± 12	47
≤ 50 years	121	53
> 50 years	134	
Sex		
Female	46	18
Male	209	82
Subsite		
Gingivo-buccal complex	162	64
Tongue	88	34
Other	5	2
Neoadjuvant Therapy		
No	217	85
Yes	38	15
Surgical margins		
Clear/close margins	246	96
Involved margin/s	9	4
Tumour stage		
T1 & T2	124	49
T3 & T4	131	51
Nodal stage		
N0	157	62
N +	98	38
AJCC TNM Stage		
Stage I & II	92	36
Stage III & IV	163	64
Grade (n = 254)		
Grade 1 / 2	175	69
Grade 3	79	31
LVI		
Absent	223	87
Present	32	13
PNI		
Absent	189	74
Present	66	26
ENE		
Absent	226	89
Present	29	11
Group A (Triple Negative)	167	66
Group B (Single Positive)	59	23
Only LVI +	10	4
Only PNI +	38	15
Only ENE +	11	4
Group C (Double Positive)	19	7
LVI + /PNI +	11	4
LVI + /ENE +	1	1
PNI + /ENE +	7	3
Group D (Triple Positive)	10	4
Adjuvant therapy	167	66
Adjuvant radiotherapy	105	41
Adjuvant chemoradiotherapy	62	24
Follow up (in months)		
Median	24 (2–95)	

Table 1 (continued)

Clinicopathological parameters	Number of patients ($n=255$)	Percentage
Recurrence	61	24
Mortality	55	22

demonstrated that advanced TNM stage (III/IV), grade 2 disease, LVI, PNI, and ENE were associated with significantly lower OS. The multivariate analysis revealed that advanced TNM stage and PNI were independent predictors of OS. The univariate and multivariate analysis of OS is described in Table 4

Analysis of the Study Groups

Among the study groups, Group A (triple negative) consisted of 167 patients (66%), Group B (single positive) had 59 patients (23%), Group C (double positive) had 19 patients (7%), and Group D (triple positive) had ten patients (4%). No significant differences among the study groups were observed in age, subsite involvement, or surgical margin status. However, there were statistically significant differences

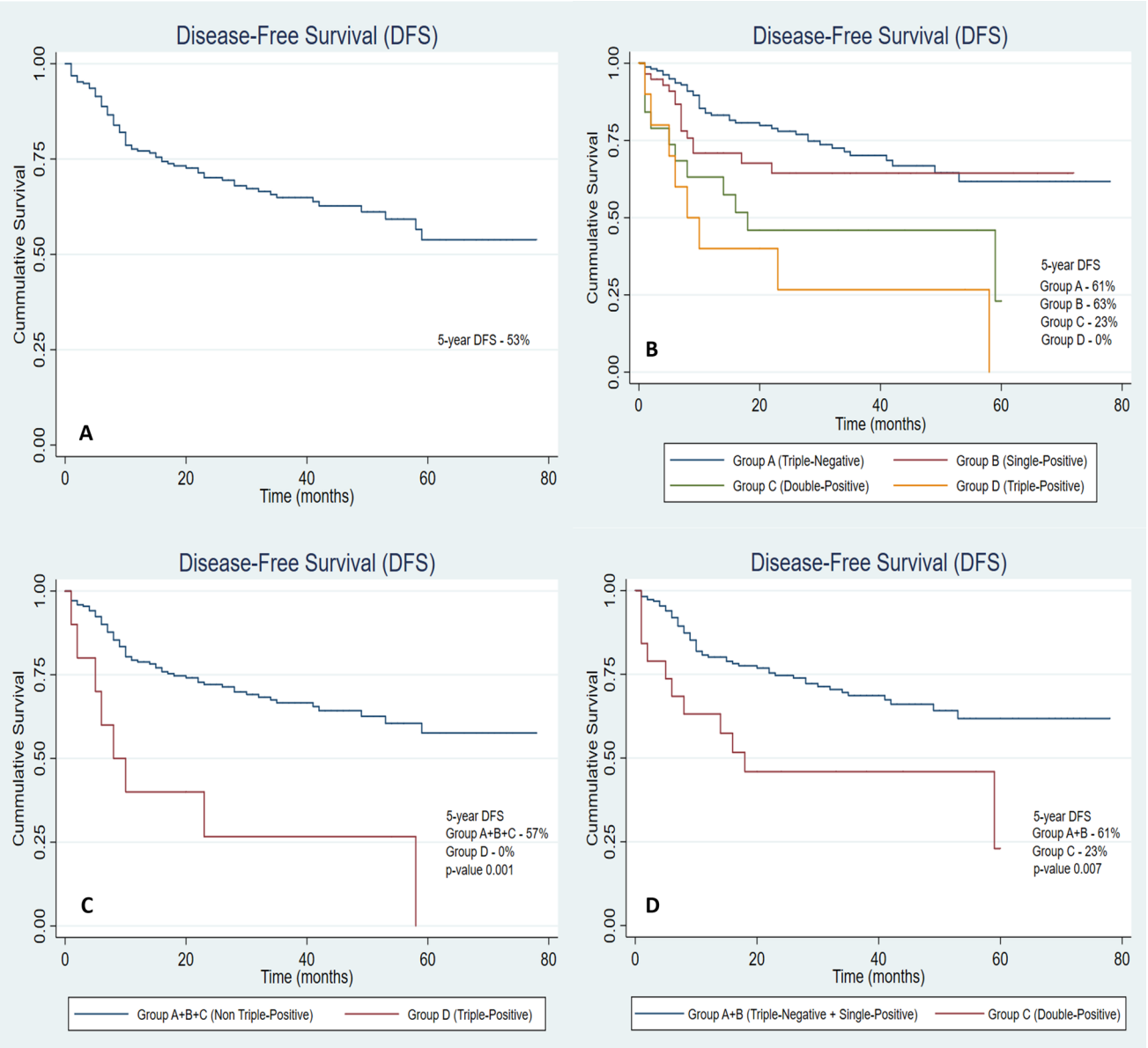

Fig. 2 Kaplan Meier Curve. **A** DFS of the total study population. **B** DFS of different study groups. **C** DFS comparison between Triple-Positive vs Non-Triple-Positive. **D** DFS comparison between Double-Positive vs Triple-Negative / Single-Positive

Table 2 Clinico-pathological parameters among Study Groups

Clinicopathological parameters	Group A ($n=167$)	Group B ($n=59$)	Group C ($n=19$)	Group D ($n=10$)	p-value	Total
Age						
(Mean ± SD)	53 ± 13	49 ± 12	54 ± 9	47 ± 9	0.351	52 ± 12
≤ 50 years	75 (45%)	31 (52%)	8 (42%)	7 (70%)		121 (47%)
> 50 years	92 (55%)	28 (48%)	11 (58%)	3 (30%)		134 (53%)
Sex						
Female	25 (15%)	14 (24%)	7 (37%)	0 (0%)	0.030	46 (18%)
Male	142 (85%)	45 (76%)	12 (63%)	10 (100%)		209 (82%)
Subsite						
Gingivo-buccal complex	112 (67%)	35 (59%)	9 (47%)	6 (60%)	0.307	162 (64%)
Tongue	50 (30%)	24 (41%)	10 (53%)	4 (40%)		88 (34%)
Other	5 (3%)	0 (0%)	0 (0%)	0 (0%)		5 (2%)
Neoadjuvant therapy						
No	148 (89%)	50 (85%)	13 (68%)	6 (60%)	0.013	217 (85%)
Yes	19 (11%)	9 (15%)	6 (32%)	4 (40%)		38 (15%)
Surgical margins						
Clear/close margins	160 (96%)	57 (96%)	19(100%)	10 (100%)	1.000	246 (96%)
Involved margin/s	7 (4%)	2 (4%)	0 (0%)	0 (0%)		9 (4%)
Tumour stage						
T1 & T2	94 (56%)	20 (34%)	8 (42%)	2 (20%)	0.005	124 (49%)
T3 & T4	73 (44%)	39 (66%)	11 (58%)	8 (80%)		131 (51%)
Nodal stage						
N0	125 (75%)	27 (46%)	5 (26%)	0 (0%)	< 0.001	157 (62%)
N +	42 (25%)	32 (55%)	14 (74%)	10 (10%)		98 (38%)
AJCC TNM stage						
Stage I & II	79 (47%)	10 (17%)	3 (16%)	0 (0%)	< 0.001	92 (36%)
Stage III & IV	88 (53%)	49 (83%)	16 (84%)	10 (10%)		163 (64%)
Grade						
Grade 1 / 2	129 (78%)	36 (61%)	8 (42%)	2 (20%)	< 0.001	175 (69%)
Grade 3	37 (22%)	23 (39%)	11 (58%)	8 (80%)		79 (31%)
LVI						
Absent	167 (100%)	49 (83%)	7 (37%)	0 (0%)	–	223 (87%)
Present	0 (0%)	10 (17%)	12 (63%)	10 (100%)		32 (13%)
PNI						
Absent	167 (100%)	21 (36%)	1 (5%)	0 (0%)	–	189 (74%)
Present	0 (0%)	38 (64%)	18 (95%)	10 (100%)		66 (26%)
ENE						
Absent	167 (100%)	48 (81%)	11 (58%)	0 (0%)	–	226 (88%)
Present	0 (0%)	11 (19%)	8 (42%)	10 (100%)		29 (12%)
Adjuvant Radiotherapy	71 (43%)	27 (44%)	7 (36%)	0 (0%)	0.032	105 (41%)
Adjuvant Chemoradiotherapy	24 (14%)	21 (36%)	8 (42%)	9 (90%)	< 0.001	62 (24%)
Median follow up	–	–	–	–	–	24 months
Recurrence	32 (19%)	13 (22%)	9 (47%)	7 (70%)	–	61 (24%)
Mortality	24 (14%)	15 (25%)	10 (53%)	6 (60%)	–	55 (22%)

in sex, neoadjuvant therapy, T stage, N stage, TNM stage, and grade of the disease. Out of ten patients in Group D, nine patients received adjuvant chemoradiotherapy. The remaining patient has received three cycles of NACT, developed nodal recurrence at three months postoperative period and was advised targeted therapy. The distribution of clinicopathological parameters and statistical analysis among them is described in Table 2.

The 5-year disease-free survival (DFS) rates were as follows: Group A—61%, Group B—63%, Group C—23%, and Group D—0% (Fig. 2B). The median DFS was not reached in Group A, and B. Group C had a median DFS of 18 months, while Group D had a median DFS of eight months. Survival analysis revealed significantly lower DFS in Group C (HR 2.70, 95%CI 1.39–5.25, p-value 0.003) and Group D (HR 4.31, 95%CI 2.01–9.20, p-value < 0.001)

Table 3 Correlation of the clinico-pathological parameters with Disease-Free Survival (DFS)

Clinicopathological parameters	5-year DFS	Univariate analysis (DFS)		Multivariate analysis (DFS)	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	–	1.00 (0.99–1.02)	0.412	–	–
Sex					
Female	44%	0.70 (0.40–1.21)	0.207	–	–
Male	55%				
Subsite					
Gingivo-buccal complex	53%	1.04 (0.65–1.64)	0.879	–	–
Tongue	51%	0.55 (0.07–4.03)	0.560		
Others	67%				
Neoadjuvant therapy					
No	59%	1.48 (0.86–2.53)	0.155	–	–
Yes	36%				
Surgical margins					
Clear margins	40%	1.06 (0.66–1.71)	0.793	–	–
Close margins		2.39 (0.94–6.05)	0.066		
Involved margin/s					
AJCC TNM stage					
Stage I & II	72%	2.93 (1.69–5.09)	**< 0.001**	2.35 (1.32–4.20)	**0.004**
Stage III & IV	42%				
Grade					
Grade 1	51%	1.49 (0.91–2.43)	0.108	1.38 (0.93–2.02)	0.101
Grade 2	55%	3.21 (1.44–7.14)	**0.004**		
Grade 3	39%				
LVI					
Absent	57%	2.22 (1.31–3.77)	**0.003**	1.18 (0.65–2.18)	0.575
Present	28%				
PNI					
Absent	60%	2.07 (1.29–3.30)	**0.002**	1.39 (0.81–2.38)	0.238
Present	31%				
ENE					
Absent	60%	2.76 (1.62–4.76)	**< 0.001**	1.51 (0.83–2.75)	0.172
Present	15%				

compared to Group A. The 5-year DFS of the triple positive group (Group D) was 0%, which was significantly lower than the non-triple positive group (Group A + B + C) with a 5-year DFS of 57% (HR 3.51, 95%CI 1.69–7.33, *p*-value 0.001) (Fig. 2C). Similarly, the 5-year DFS of the double-positive group (Group C) was significantly lower (HR 2.44, 95%CI 1.28–4.65, *p*-value 0.007) compared to the single-positive or triple-negative study population (Group A + B), with a 5-year DFS of 23% versus 61% (Fig. 2D). Detailed results on the correlation of different study groups with DFS are provided in Table 5

The 5-year OS of Group A, B, C and D was 78%, 63%, 50% and 0%, respectively (Fig. 3B). The median DFS was not reached in Group A and B. The median OS of Group C was 38 months, while for Group D, it was 24 months. Survival analysis revealed significantly lower OS in Group B (HR 2.59, 95%CI 1.35–4.98, *p*-value 0.004), Group C (HR 4.06, 95%CI 1.93–8.54, *p*-value < 0.001), and Group D (HR 5.61, 95%CI 2.29–13.91, *p*-value < 0.001) compared to Group A. The 5-year OS of the triple positive group was 0%, significantly lower than the non-triple positive group with a 5-year OS of 72% (HR 3.67, 95%CI 1.56–8.58, *p*-value 0.003) (Fig. 3C). Similarly, the 5-year OS of the double-positive group (Group C) was significantly lower (HR 3.08, 95%CI 1.54–6.19, *p*-value 0.002) compared to the single-positive or triple-negative study population (Group A + B) with a 5-year OS of 50% versus 75% (Fig. 3D). Detailed results on the correlation of different study groups with OS are provided in Table 5.

Discussion

The AJCC TNM staging for OSCC provides general information and a brief overview regarding the possible treatment and its outcomes. Several other microscopic features affect the prognosis of OSCC, which are not included in the AJCC TNM staging. The recent 8th AJCC edition has included

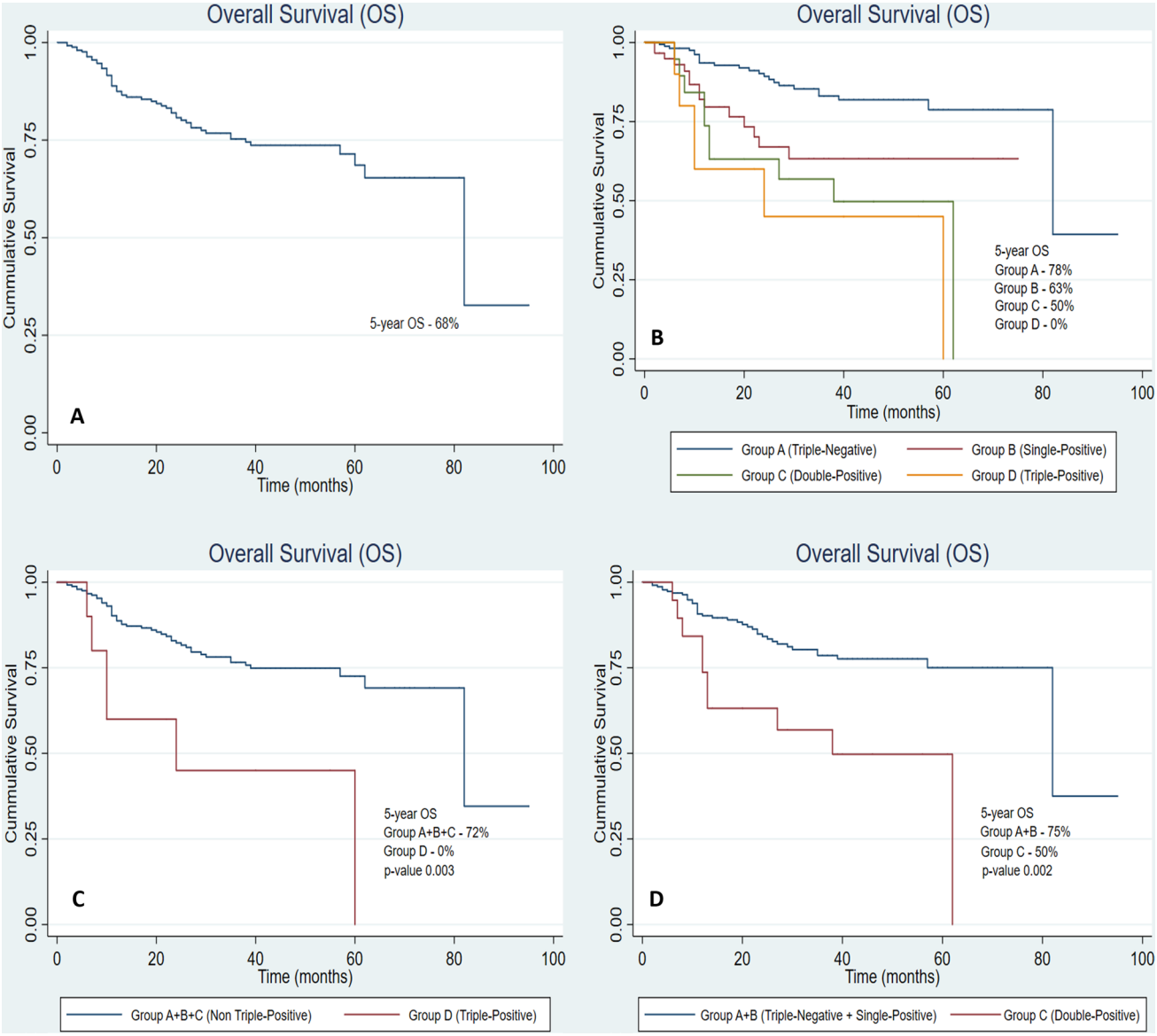

Fig. 3 Kaplan Meier Curve. **A** OS of the total study population. **B** OS of different study groups. **C** OS comparison between Triple-Positive vs Non-Triple-Positive. **D** OS comparison between Double-Positive vs Triple-Negative/Single-Positive

ENE in the TNM staging after a multicentric study showed that the presence of ENE leads to inferior disease-free survival [11, 12]. However, other proven adverse features like LVI and PNI are yet to be included. Many patients with OSCC have a combination of two or more adverse histopathological features. Even though such a combination has reported poorer survival outcomes, the standard guidelines do not clearly state the need for adjuvant therapy or the intensification of adjuvant therapy. Hence, this study was conducted to assess the effect of triple positive disease (presence of three adverse histopathological factors (LVI, PNI, ENE) on the survival outcomes in OSCC and a possible future guide to treatment in this subset of patients.

In the current study, a total of 255 patients were included in the final analysis. The study's LVI, PNI and ENE positivity was 13%, 26% and 11%, respectively. There were 167 triple-negative patients (66%), while ten patients (4%) had triple-positive disease. The single-positive group had 59 patients (23%), and the double-positive group had 19 (7%). The triple-positive group had lower DFS than non-triple-positive (0 vs 57%, *p*-value 0.001) and lower OS (0% vs 72%, *p*-value 0.003). The median DFS and OS of the triple-positive group were eight months and 24 months, respectively. Similarly, the double-positive group also had statistically significant inferior DFS (23% vs 67%, *p*-value 0.007) and OS (50% vs 75%, *p*-value 0.002) compared to the

Table 4 Correlation of the clinico-pathological parameters with Overall Survival (OS)

Clinicopathological parameters	5-year OS	Univariate analysis (OS)		Multivariate analysis (OS)	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Age	–	1.01 (0.99–1.03)	0.279	–	–
Sex					
Female	50%	0.57 (0.30–1.08)	0.086	–	–
Male	72%				
Subsite					
Gingivo-buccal complex	65%	0.97 (0.56–1.70)	0.940	–	–
Tongue	64%	0.89 (0.12–6.55)	0.914		
Others	66%				
Neoadjuvant therapy					
No	71%	1.31 (0.72–2.62)	0.324	–	–
Yes	58%				
Surgical margins					
Clear margins	74%	1.29 (0.74–2.24)	0.374	–	–
Close margins	60%	1.03 (0.51–5.58)	0.386		
Involved margin/s	61%				
AJCC TNM stage					
Stage I & II	89%	5.04 (2.28–11.15)	**< 0.001**	3.83 (1.68–8.74)	**0.001**
Stage III & IV	56%				
Grade					
Grade 1	71%	1.85 (1.04–3.28)	**0.033**	1.34 (0.85–2.13)	0.204
Grade 2	62%	2.62 (0.98–7.00)	0.054		
Grade 3	54%				
LVI					
Absent	74%	2.23 (1.19–4.16)	**0.012**	0.88 (0.43–1.80)	0.729
Present	37%				
PNI					
Absent	76%	3.12 (1.82–5.34)	**< 0.001**	2.12 (1.13–3.98)	**0.019**
Present	44%				
ENE					
Absent	74%	3.73 (2.07–6.71)	**< 0.001**	1.66 (0.85–3.21)	0.134
Present	33%				

single-positive/triple-negative group. The median DFS and OS of the double-positive group were 18 and 38 months, respectively.

In only a previous study, Lin et al. analysed 554 OSCC patients, out of which 15 (2.7%) had triple-positive disease. They reported 5-year OS of 20% in the triple-positive group, while our study reported 0%. This discrepancy might be attributed to a higher proportion of lymph node positive (38% vs 26%) and stage III/IV patients (53% vs 46%) in our study [14]. Few studies in the current literature have evaluated the combination of LVI and PNI in OSCC. The double-positive rate in a study by Huang et al. was 4.7%, which had a median survival of mere ten months and was significantly lower compared to other patients [9]. In another study, Ting et al. reported a 27.6% double-positivity rate, which was higher than the current study as they only included T3-T4 patients [10]. Despite the variations in rates, all three studies, including the current one, consistently demonstrated poor survival outcomes in the double-positive patient subsets.

In a recent systematic review and metanalysis, Dolens et al. investigated the impact of histopathological features like LVI, PNI, ENE, DOI, surgical margins and bone invasion on the prognosis of OSCC by reviewing 172 previous studies from 1999 to 2021. A total of 30 studies evaluating LVI, 45 studies on PNI and 31 studies on ENE were included in the analysis. All three histopathological factors were associated with significantly lower DFS, disease-specific survival (DSS) and OS (p-value < 0.001) [2]. In our study, LVI, PNI and ENE showed significantly lower DFS and OS on univariate analysis. The PNI also showed a correlation with OS on multivariate analysis. A multi-institutional analysis of 557 patients has shown that adding adjuvant radiotherapy to PNI + tumours improved DFS [6]. Similarly, a population-based analysis of nearly 17,000 patients reported that patients with LVI + had OS benefit with addition adjuvant radiotherapy or chemoradiotherapy [7]. In contrast, the literature suggests that these risk factors' presence does not warrant adjuvant treatment [18–21]. Hence, the guidelines

Table 5 Analysis of DFS and OS among different Study Groups

	5-year DFS	Median DFS	HR (95%CI)	*p*-value
Study groups				
Group A	61%	Not reached	(Reference)	0.143
Group B	63%	Not reached	1.52 (0.86–2.68)	**0.003**
Group C	23%	18 months	2.70 (1.39–5.25)	**< 0.001**
Group D	0%	8 months	4.31 (2.01–9.20)	
Group A + B + C				
(Non-Triple-Positive)	57%	Not reached	(Reference)	**0.001**
Group D (Triple-Positive)	0%	8 months	3.51 (1.69–7.33)	
Group A + B				
(Triple-Negative + Single-Positive)	61%	Not reached	(reference)	**0.007**
Group C (Double-Positive)	23%	18 months	2.44 (1.28–4.65)	
	5-year OS	**Median OS**	**HR (95%CI)**	***p*-value**
Study groups				
Group A	78%	Not reached	(Reference)	0.004
Group B	63%	Not reached	2.59 (1.35–4.98)	< 0.001
Group C	50%	38 months	4.06 (1.93–8.54)	< 0.001
Group D	0%	24 months	5.61 (2.29–13.91)	
Group A + B + C				
(Non-Triple-Positive)	72%	82 months	(Reference)	0.003
Group D (Triple-Positive)	0%	24 months	3.67 (1.56–8.58)	
Group A + B				
(Triple-Negative + Single-Positive)	75%	82 months	(Reference)	0.002
Group C (Double-Positive)	50%	38 months	3.08 (1.54–6.19	

have not yet established the definitive indication of adjuvant radiotherapy/chemoradiotherapy in LVI + or PNI + [22]. Thus, we need to identify and study survival outcomes in subgroups of patients with a combination of two or more risk factors.

In an earlier study, Chen and colleagues tried identifying the high-risk sub-groups of OSCC that may benefit from adjuvant therapy. They reported that the patients with two minor risk factors (pT4, N + , LVI, PNI, close margins, depth ≥ 10 mm, grade 3) benefited from adjuvant radiotherapy. Patients with three or more risk factors derived survival benefits after receiving adjuvant chemoradiotherapy [5]. A systematic review and meta-analysis of 12 studies have shown that adjuvant chemoradiotherapy significantly improved OS (p-value < 0.001) solely in the presence of multiple minor pathological risk factors [23]. In contrast, a study by Chen et al. showed no survival benefit of adding adjuvant radiotherapy in double-positive (LVI + /PNI +) early-stage (stage I/II) OSCC [24]. Hence, it would be beneficial to develop and validate risk assessment models incorporating various prognostic factors to identify high-risk groups of patients in whom intensification of adjuvant treatment would be beneficial.

The management of locally advanced oral squamous cell carcinoma (OSCC) typically involves a multimodality approach, which includes surgery followed by adjuvant radiotherapy/ chemoradiotherapy, with or without neoadjuvant chemotherapy. Unlike breast cancer, maintenance adjuvant therapy is not yet established as the standard of care for high-risk OSCC. In the current study, the triple-positive patients had a dismal 5-year DFS and OS of 0% and 0%, respectively, despite receiving the recommended treatment regimen. Hence, there is a growing need to check for genetic mutation/biomarkers in this subset of patients. Artificial intelligence and machine learning can be used to identify molecular biomarkers. This identification of biomarkers will help prescribe personalised, targeted therapy to enhance the survival of double/triple-positive disease [25, 26].

The biology of OSCC has many uncertainties due to a complex tumour microenvironment. Understanding the complex relationship between different components of tumour microenvironment has led to newer treatment modalities like targeted therapy, immunotherapy, etc. [27]. Maintenance metronomic therapy using oral methotrexate and erlotinib or oral tegafur-uracil have shown to downstage disease and prevent relapse in non-metastatic locally advanced OSCC [28–32]. However, these studies are limited by their small sample sizes, single-centre designs, and retrospective analysis. Few ongoing trials in intermediate and high-risk OSCC assess the role of chemotherapy, radiotherapy

and different targeted therapy [33]. Currently, the role of an immune checkpoint inhibitor (Pembrolizumab) is being investigated in a clinical trial as a maintenance therapy in locally advanced OSCC [34]. Further, randomised clinical trials, nomograms and risk assessment models are required to optimise the treatment of high-risk patients and improve their oncological outcomes.

Strength and Limitations

The current study provided insights into the survival outcomes of oral squamous cell carcinoma patients with a combination of adverse histopathological features. The current study focused on triple-positive disease (LVI + / PNI + /ENE +), which has been inadequately explored in the existing literature. The limitation of the study remains a single-centre, retrospective data. Moreover, the segregation between invasion of lymphatics or blood vessels was not attempted, which have been identified as two different risk factors [22, 35]. Other parameters which have impact on survival outcomes like performance status, Charlson comorbidity index and DOI are not studied here. Furthermore, we acknowledge that the traditional pathological prognosticators in OSCC like positive surgical and ENE have not been correlated with DFS and OS in the current study. We observed that only AJCC staging showed significant correlation with DFS in multivariate analysis. Similarly, PNI was a better prognostic marker of OS than positive margin and ENE. The following contradictory results to the current understanding of OSCC may be due to improper patient grouping and sample size limitation. However, the analysis was done from a prospectively maintained oral cancer database, and all consecutive patients were included in the study. To validate our findings, further investigations incorporating larger sample sizes, preferably prospective in nature, are necessary.

Conclusion

The triple-positive disease, characterised by the presence of lymphovascular invasion (LVI +), perineural invasion (PNI +), and extranodal extension (ENE +), had demonstrated inferior outcomes, with no patients achieving disease-free survival or overall survival at the 5-year follow-up. The combination of adverse histopathological features should be taken into consideration while planning adjuvant therapy for such patients. The presence of multiple adverse factors necessitates modification of adjuvant therapy and therapeutic strategy, which may enhance survival outcomes. There is also a pressing need for innovative therapeutic approaches to enhance outcomes in this patient population.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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