CHAPTER 4

RESULTS

4.1. Introduction

This cross-sectional study is based on fifty OSCC paraffin embedded tissue samples of surgical specimens obtained from the archives of the Department of Oral Pathology, Oral Medicine and Periodontology and the Oral Pathology Diagnostic Laboratory. Equal number of tongue and buccal mucosa OSCC were included. The related data was obtained from the Malaysian Oral Cancer Database and Tumor Bank System (MOCDTBS) coordinated by the Oral Cancer Research Coordinating Center (OCRCC). PASW 18.0 software was used to conduct the descriptive, survival and inferential statistical analysis.

4.1.1. FISH technique evaluation criteria:

Evaluation of the preparation was performed by counting at least 200 nuclei per slide, according to criteria described by Hopman et al. 1988.

4.1.2. Image analysis:

Enumeration of the florescent signals was performed in at least 200 nuclei per slide under objective power of 100X, using an Olympus florescent microscope BX61 equipped with single band sets for DAPI, Fluorescein Isothiocyanate (FITC) and spectrum Orange to discriminate the color signals of green for chromosome 11 centromeric DNA and orange for *CCND1* during scoring. Images for documentation were then captured using a spectral imaging camera and processed case data manager expo 5.0. (Figure 4.1)

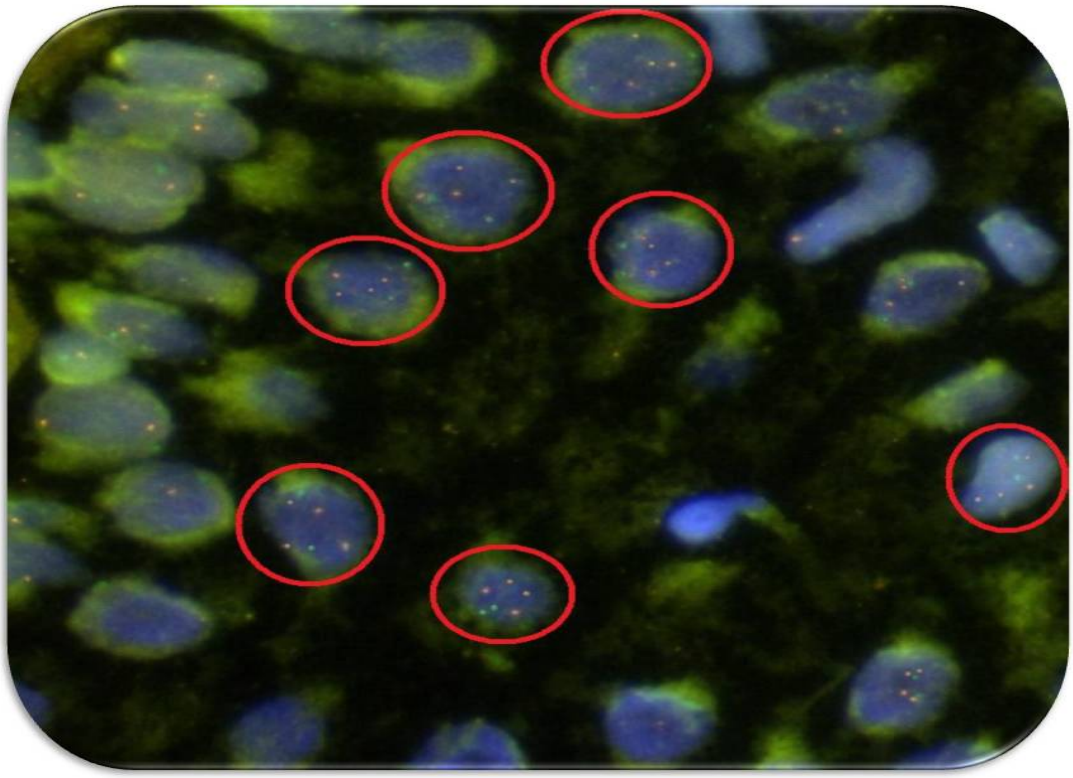

Figure 4.1 FISH staining showing green (chromosome 11) and orange (Cyclin D1) signals and amplification ratio in nuclei marked by the red circles.

4.2. Sociodemographic characteristic

This study was conducted on samples from19 males (38%) and 31 females (62%). The table below provide the total number of patients with oral squamous cell carcinoma according to gender, where we can find the majority of the patients were Indians followed by Malay then Chinese. Indian females are the largest group in this study. (Table 4.1)

The age of the patients ranged from 26-94 years with mean of 60 years old (Table 4.2). Majority of patients (56%) were more than or equals to 60 years old.

Table 4.1 Demographic distribution according to gender and ethnicity

Gender/ Ethnicity	I n (%)	M n (%)	C n (%)	Total n (%)
Male	10 (29.4%)	4 (40%)	5 (83.3%)	19 (38%)
Female	24 (70.6%)	6 (60%)	1 (16.7%)	31 (62%)
Total	34 (68%)	10 (20%)	6 (12%)	50 (100%)

I=Indian M=Malay C=Chinese

n=Number

Table 4.2 Distribution of cases according to age

Demographic parameters			
Age (Years)	Tongue n (%)	Buccal mucosa n (%)	Total n (%)
<60 yrs	9(36%)	13(52%)	22 (100%)
≥60 yrs	16(64%)	12(48%)	28 (100%)

4.3. Clinicopathological features

4.3.1. Tumor site:

The current study include half the number of total cases (n=50) from tongue SCC 25 (50%) and the other half from buccal mucosa SCC 25(50%). (Table 4.3)

4.3.2. Broder's classification

Twenty cases (40%) were graded as well-differentiated OSCC in this study. Moderately differentiated OSCC was seen in 26(52%) and poorly differentiated OSCC in 4 (8%). (Table 4.3)

4.3.3 Pattern of invasion

In this study majority of patients 44(88%) had non-cohesive type (type 3+4) pattern of invasion. Only six (12%) of cases had cohesive type (type 1+2) invasion of the tumor front. (Table 4.3)

4.3.4. Tumor size (pT)

In this study, histopathological features such as greatest tumor dimension (T), lymph node metastasis (N) and pathological TNM staging (pTNM) were studied. More than half (56%) had greatest tumor dimension more than 2 cm but not more than 4 cm in greatest dimension. Five cases had tumor size of 2 cm or less in greatest dimension, where as 13 samples were more than 4 cm in greatest dimension. Four cases had the tumor size more than 4 cm with invasion into adjacent structures. (Table 4.3)

4.3.5. Lymph node metastasis

Twenty seven samples in the study showed positive lymph node metastasis.

4.3.6. pTNM stage

In the current study, the pathological TNM staging was grouped into early stage (I+II) which represented 36% and advanced stage (III+IV) which presented the majority of cases 64%. (Table 4.3)

4.3.7 Tumor depth

In addition to pTNM staging, the tumor depth was also evaluated. Almost all cases had tumor depth of less than 5 mm or more. (Table 4.3)

Table 4.3 Clinicopathological features

Parameters	Tumor Site		
Histopathological grade	Tongue n (%)	Buccal Mucosa n (%)	Total n (%)
Well	8(32%)	12(48%)	20 (40%)
Moderately	13(52%)	13(52%)	26 (52%)
Poorly	4(16%)	0(0%)	4 (8%)
Pattern of invasion			
Cohesive	1(4%)	5(20%)	6 (12%)
Non-cohesive	24(96%)	20(80%)	44 (88%)
Tumor size (pT)			
pT≤ 2 cm	3(12%)	2(8%)	5 (10%)
2 < pT ≤ 4cm	12(48%)	16(64%)	28 (56%)
pT > 4cm	9(36%)	4(16%)	13 (26%)
Any pT with invasion	1(4%)	3(12%)	4 (8%)
lymph node metastasis(pN)			
N0	14(56%)	9(36%)	23 (46%)
N+ve	11(44%)	16(64%)	27 (54%
pTNM stage			
Stage I + II (early)	10(40%)	8(32%)	18 (36%)
Stage III + IV (advanced)	15(60%)	17(68%)	32 (64%)
Tumor Depth			
< 5 mm	2(8%)	1(4%)	3 (6%)
≥ 5 mm	23(92%)	24(96%)	47 (94%)
Total	25(50%)	25(50%)	50 (100%)

N0= no regional lymph node metastasis

N+ve= Positive regional lymph node metastasis

4.4. Cyclin D1 amplification:

Cyclin D1 amplification was positive in 36 out of 50 cases and more than half of the positive samples were tongue SCC. (Table 4.4)

Table 4.4 cyclin D1 amplification

Cyclin D1 amplification	Tongue	Buccal Mucosa	Total n (%)
Positive	22(88%)	14(56%)	36 (72%)
Negative	3(12%)	11(44%)	14 (28%)
Total	25(50%)	25(50%)	50 (100%)

4.4.1. Association between sociodemographic characteristic and cyclin D1 amplification:

4.4.1.1. Association between age and cyclin D1 amplification based on tumor site:

Fisher Exact test of independence was used to determine if cyclin D1 amplification is dependent on age. Given $\alpha = 0.05$, the results suggest lack of dependency. No significant difference between age and cyclin D1 amplification in tongue and buccal mucosa SCC (tongue *p* value=0.287, buccal mucosa *p* value= 0.163) was noted. (Table 4.5)

Table 4.5 Association between age and cyclin D1 amplification

Tumor Site		**cyclin D1 amplification**		
	Age	**Positive**	**Negative**	***p*-value**
		n (%)	**n (%)**	
Tongue	< 60yrs	7(31.8%)	2(66.7%)	0.287**
	≥ 60yrs	15(68.2%)	1(33.3%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	< 60yrs	9(64.3%)	4(36.4%)	0.163*
	≥ 60yrs	5(35.7%)	7(63.6%)	
Total n (%)		14(100%)	11(100%)	

*Chi-square test was used.

**Fisher exact test was used.

4.4.1.2. Association between gender and cyclin D1 amplification based on tumor site:

A 2X2 Fisher Exact test of independence was used to determine if cyclin D1 amplification is dependent on gender for each tumor sites. Given $\alpha = 0.05$, the results suggest lack of dependency, tongue *p*-value= 0.469 and buccal mucosa *p*-value=0.102 (Table 4.6). Although amplification of cyclic D1 is more significantly by demonstrated in tongue tumor, gender has no influence on the amplification of cyclin D1.

Table 4.6 Association between gender and cyclin D1 amplification based on tumor site

Tumor Site		**cyclin D1 amplification**		
	Gender	**Positive n (%)**	**Negative n (%)**	***p*-value**
Tongue	Male	10(45.5%)	2(66.7%)	0.469*
	Female	12(54.5%)	1(33.3%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	Male	2(14.3%)	5(45.5%)	0.102*
	Female	12(85.7%)	6(54.5%)	
Total n (%)		14(100%)	11(100%)	

*Fisher exact test was used.

4.4.1.3. Association between ethnicity and cyclin D1 amplification based on tumor site

The results suggest an insignificant association between ethnicity and cyclin D1 amplification in tongue and buccal mucosa (tongue *p*-value=0.830, buccal mucosa *p*-value=0.072). (Table 4.7)

Table 4.7 Association between ethnicity and cyclin D1 amplification based on tumor site

Tumor Site		**cyclin D1 amplification**		
	Ethnicity	**Positive**	**Negative**	***p*-value**
		n (%)	**n (%)**	
Tongue	Malay	6(27.3%)	1(33.3%)	0.830*
	Indian	11(50%)	1(33.3%)	
	Chinese	5(22.7%)	1(33.3%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	Malay	0(0.0%)	3(27.3%)	0.072**
	Indian	14(100%)	8(72.7%)	
	Chinese	-	-	
Total n (%)		14(100%)	11(100%)	

*Chi-square test was used.

**Fisher exact was used.

4.4.2. Association between clinicopathological features and cyclin D1 amplification:

4.4.2.1. Association between Tumor site and Cyclin D1 amplification (Tongue and Buccal Mucosa SCC)

A 2X2 chi-square test of independence was used to determine if cyclin D1 amplification is dependent on tumor site. Given $\alpha = 0.05$, the results suggest dependency, $\chi 2$ (one, N = 50) = 6.349. A significant result was observed between cyclin D1 amplification and SCC of tongue and buccal mucosa with cyclin D1 amplification positive in 22 of tongue SCC cases whereas only in 14 of buccal mucosa SCC cases were positive. (*p*-value=0.012). (Table 4.8)

Table 4.8 Association between the Amplification of Cyclin D1 and Tumor Sites (Buccal Mucosa and Tongue SCC)

Tumor Site	Cyclin D1 amplification				
	Positive n (%)	**Negative** n (%)	**Total**	χ^2	**P-value**
Tongue	22 (88%)	3 (12%)	25(100%)	6.349	0.012*
Buccal Mucosa	14 (56%)	11 (44%)	25(100%)		

*Chi-square test was used.

4.4.2.2. Association between modified Broder's grading and cyclin D1 amplification based on tumor site:

No statistical significant result was observed between cyclin D1 amplification and modified Broder's grading in tongue and buccal mucosa SCC. (Tongue *p*-value=0.157, buccal mucosa *p*-value=0.163). (Table 4.9)

Table 4.9 Association between modified Broder's grading and cyclin D1 amplification based on tumor site

Tumor Site		**cyclin D1 amplification**		
	Modified Broder's grading	**Positive n (%)**	**Negative n (%)**	***p*-value**
Tongue	Well	6(75%)	2(25%)	0.157*
	Moderately	12(92.3%)	1(7.7%)	
	Poorly	4(100%)	0(0.0%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	Well	5(41.7%)	7(58.3%)	0.163*
	Moderately	9(69.2%)	4(30.8%)	
	Poorly	-	-	
Total n (%)		14(100%)	11(100%)	

*Chi-square test was used.

4.4.2.3. Association between pattern of invasion and cyclin D1 amplification based on tumor site:

In this analysis, the association between cyclin D1 amplification and pattern of invasion was performed based on tumor sites (Tongue and Buccal mucosa). No significant result was observed between cyclin D1 amplification and the pattern of invasion at the invasive front in both tongue and buccal mucosa SCC. (Table 4.10)

Table 4.10 Association between pattern of invasion and cyclin D1 amplification based on tumor site

Tumor Site		**cyclin D1 amplification**		
	Pattern of invasion	**Positive n (%)**	**Negative n (%)**	***p*-value**
Tongue	COHESIVE	1(4.5%)	0(0.0%)	0.880*
	NON-COHESIVE	21(95.5%)	3(100%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	COHESIVE	1(7.1%)	4(36.4%)	0.096*
	NON-COHESIVE	13(92.9%)	7(63.6%)	
Total n (%)		14(100%)	11(100%)	

*Fisher-exact test was used.

4.4.2.4. Association between tumor greatest dimension (pT) and cyclin D1 amplification:

In this analysis, the association between cyclin D1 amplification and tumor greatest dimension (pT) was studied in both tongue and buccal mucosa. It is found that tumor more than 4cm in greatest dimension of tumor with invasion demonstrate cyclin D1 amplification.

However, in this study only tongue SCC cases shown a statistically significant association between of tumor invasion with positive amplification of cyclin D1. (Tongue *p*-value=0.019, Buccal mucosa *p*-value=0.267). (Table 4.11)

Table 4.11 Association between tumor greatest dimension (pT) and cyclin D1 amplification

Tumor Site		cyclin D1 amplification		
	pT	**Positive n (%)**	**Negative n (%)**	***p*-value**
Tongue	pT≤ 2 cm	1(4.5%)	2(66.7%)	0.019*
	2 < pT ≤ 4cm	11(50%)	1(33.3%)	
	pT > 4cm	9(40.9%)	0(0.0%)	
	Any pT with invasion	1(4.5%)	0(0.0%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	pT≤ 2 cm	1(7.2%)	1(9.1%)	0.267*
	2 < pT ≤ 4cm	7(50%)	9(81.8%)	
	pT > 4cm	3(21.4%)	1(9.1%)	
	Any pT with invasion	3(21.4%)	0(0.0%)	
Total n (%)		14(100%)	11(100%)	

*Chi-square was used.

4.4.2.5. Association between lymph node metastasis (pN) and cyclin D1 amplification

Twenty-seven cases had regional lymph node metastasis, out of which 21 cases demonstrated positive cyclin D1 amplification. However, 15 cases without regional lymph node metastasis also showed cyclin D1 amplification. Therefore, no statistically significant result was observed. (Table 4.12)

Table 4.12 Association between lymph node metastasis (pN) and cyclin D1 amplification

Tumor Site		**cyclin D1 amplification**		
	pN	**Positive**	**Negative**	***p*-value**
		n (%)	**n (%)**	
Tongue	N0	12(54.5%)	2(66.7%)	0.593*
	N+ve	10(45.5%)	1(33.3%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	N0	3(21.4%)	6(54.5%)	0.098*
	N+ve	11(78.6%)	5(45.5%)	
Total n (%)		14(100%)	11(100%)	

*Chi-square test was used

4.4.2.6 Association between pathological TNM staging (pTNM) and cyclin D1 amplification based on tumor site

The association between cyclin D1 amplification and pathological TNM staging (pTNM) (Stage I + II and Stage III + IV) was insignificant for both tumor sites. The test was used to analyze the data ($\alpha = 0.05$). (Table 4.13)

Table 4.13 Association between pathological TNM staging (pTNM) and cyclin D1 amplification based on tumor site

Tumor Site		**cyclin D1 amplification**		
	pTNM	**Positive**	**Negative**	***p*-value**
		n (%)	**n (%)**	
Tongue	Stage I + II(early)	8(36.4%)	2(66.7%)	0.346*
	Stage III + IV (advanced)	14(63.3%)	1(33.3%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	Stage I + II(early)	3(21.4%)	5(45.5%)	0.199*
	Stage III + IV (advanced)	11(78.6%)	6(54.5 %)	
Total n (%)		14(100%)	11(100%)	

*Fisher exact test was used.

4.4.2.7. Association between tumor depth and cyclin D1 amplification based on tumor site

In this study the association between the cyclin D1 amplification and tumor depth (less than 5 mm and more than or equal to 5 mm) was studied for both tongue and buccal mucosa SCC. A significant result was observed between cyclin D1 amplification and tumor depth in tongue SCC whereas no significant was observed in buccal mucosa SCC, (Tongue *p*-value=0.010, buccal mucosa *p*-value=0.440) (Table 4.14).

Table 4.14 Association between tumor depth and cyclin D1 amplification based on tumor site

Tumor Site		**cyclin D1 amplification**		
	Tumor depth	**Positive**	**Negative**	***p*-value**
		n (%)	**n (%)**	
Tongue	< 5 mm	0(0.0%)	2(66.7%)	0.010*
	≥ 5 mm	22(100%)	1(33.3%)	
Total n (%)		22(100%)	3(100%)	
Buccal Mucosa	< 5 mm	0(0.0%)	1(9.1%)	0.440*
	≥ 5 mm	14(100%)	10(90.9%)	
Total n (%)		14(100%)	11(100%)	

***Fisher exact test was used.**

4.5.1. Kaplan-Meier survival analysis (KMSA)

The survival rate was 47.2% for cyclin D1 amplification positive patients and 57.1% for negative patients(Fig 4.1). According to Log Rank (Mantel-Cox) test, the survival rate is significantly different (p=0.009) between positive and negative patients. (Table 4.15)

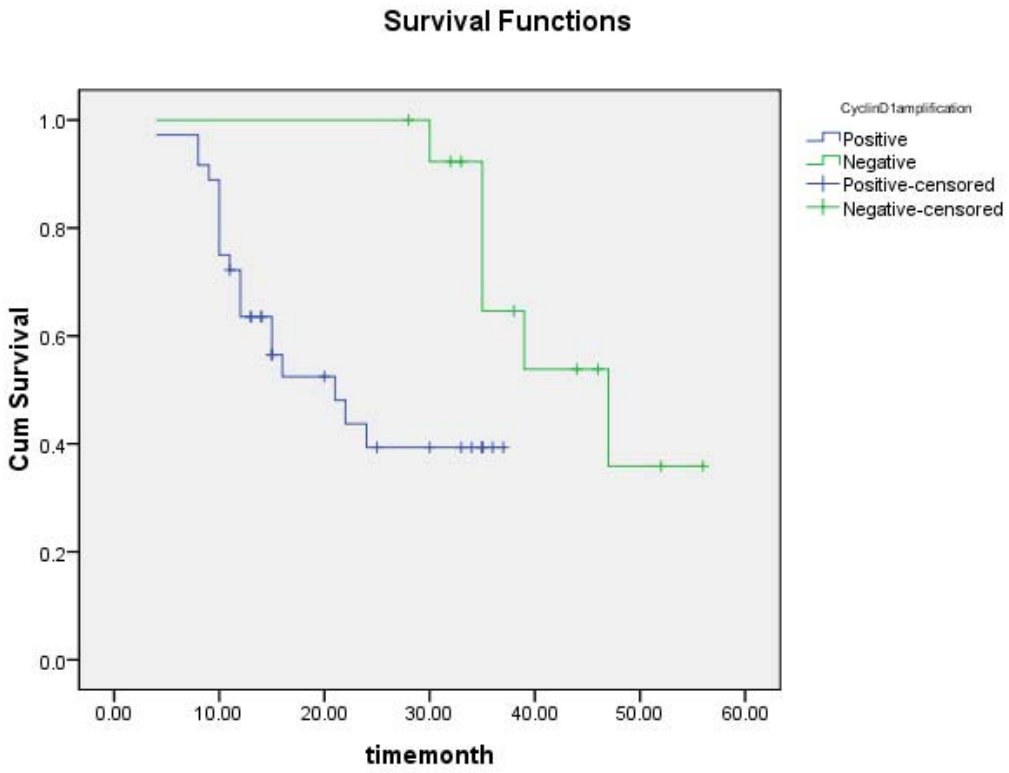

Figure 4.2 Survival rate for the sample (n=50) in relation with Cyclin D1.

Table 4.15 Log rank test to compare between survival rates between positive and negative cyclin D1 amplification.

Log Rank (Mantel-Cox)	Chi-Square	Df	*p*-value
Over all Sample	15.938	1	$p<0.001$

4.5.2. Kaplan-Meier survival analysis (KMSA) based on tumor site

The survival rate was 60% for tongue SCCand 40% for buccal mucosa SCC. According to Log Rank (Mantel-Cox) test, the survival rate is not significantly different (*p*-value=0.408) between tongue and buccal mucosa SCC patients. (Fig 4.2)

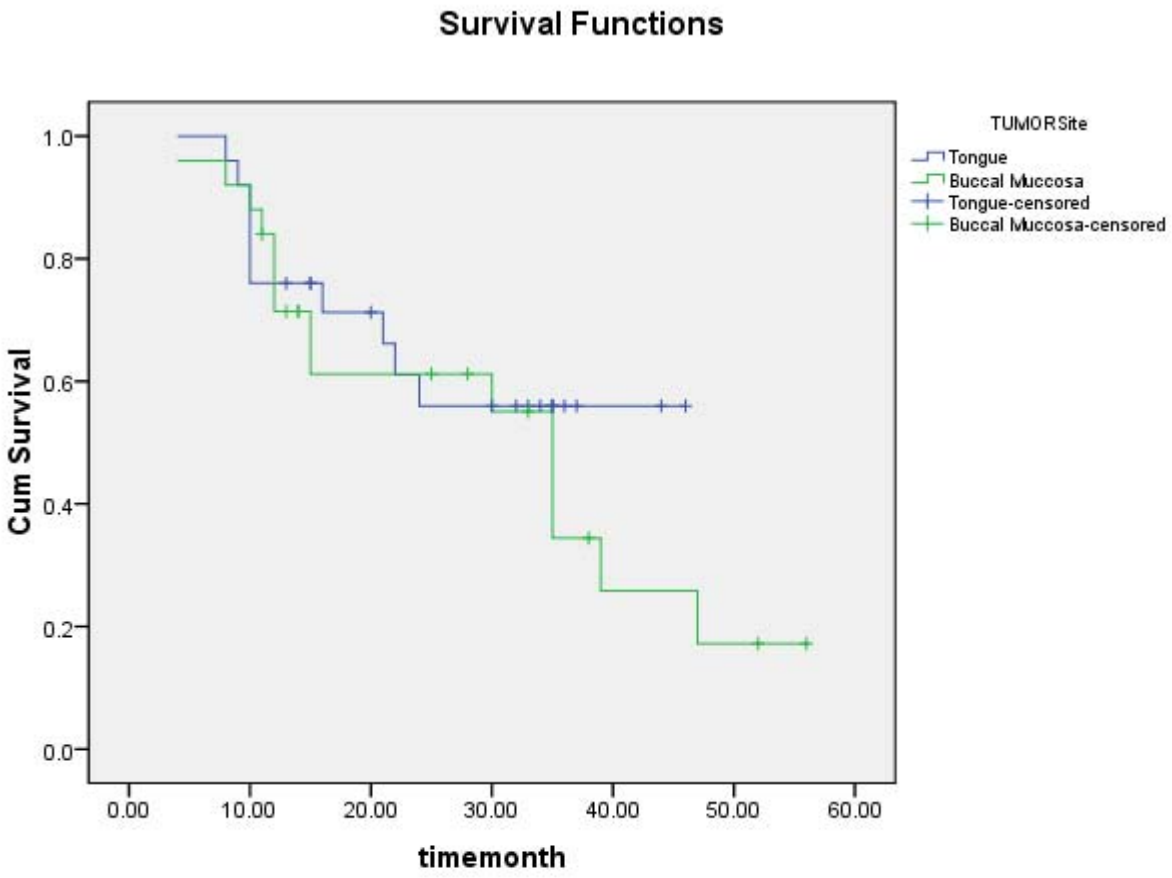

Figure 4.3 Survival rate of patients according to tumor site.

4.5.3. Kaplan-Meier survival analysis (KMSA) of cyclin D1 amplification in Tongue SCC:

According to Log Rank (Mantel-Cox) test the survival rate was not significantly different (*p*-value=0.147) for cyclin D1 amplification in tongue SCC patients. (Fig 4.3)

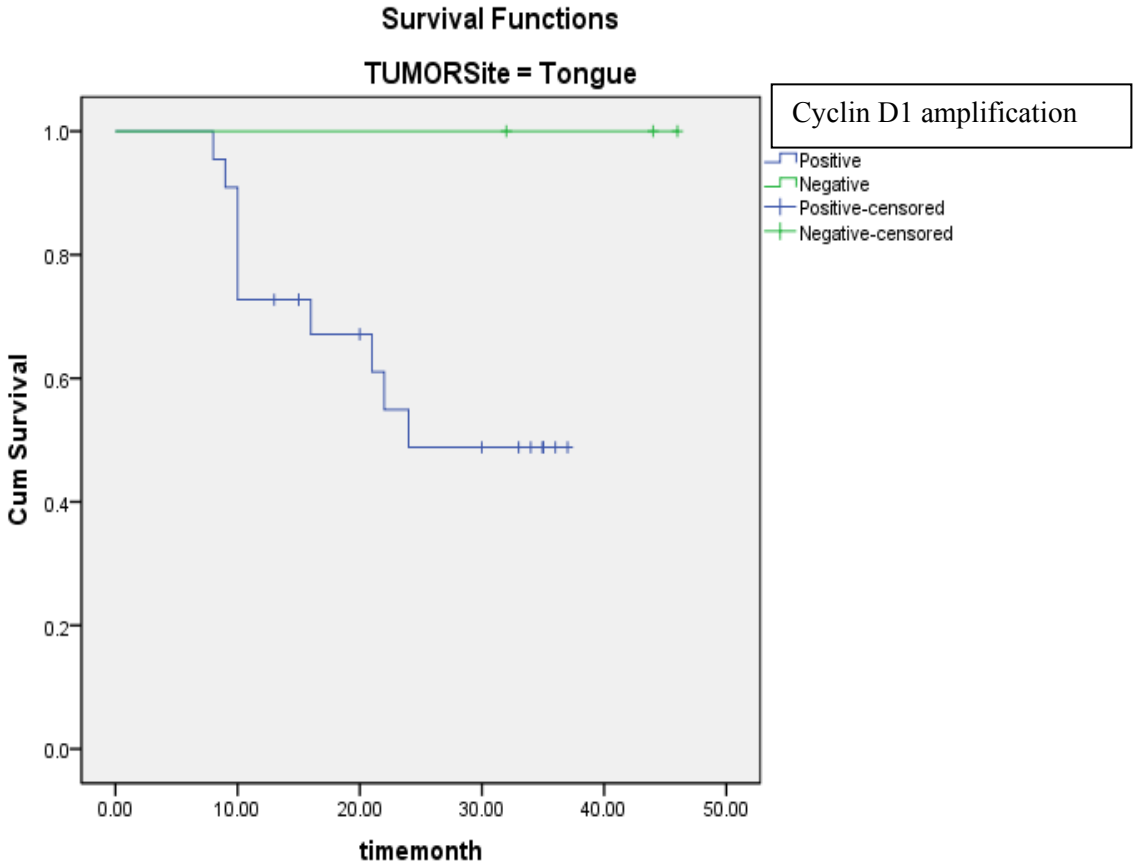

Figure 4.4 Survival rate of patients with tongue cancer (n=25) in relation to Cyclin D1 amplification.

4.5.4. Kaplan-Meier survival analysis (KMSA) of cyclin D1 amplification in Buccal mucosa SCC:

According to Log Rank (Mantel-Cox) test the survival rate was significantly different for cyclin D1 negatively amplified in buccal mucosa SCC patients . (*p*-value<0.001) (Fig 4.4)

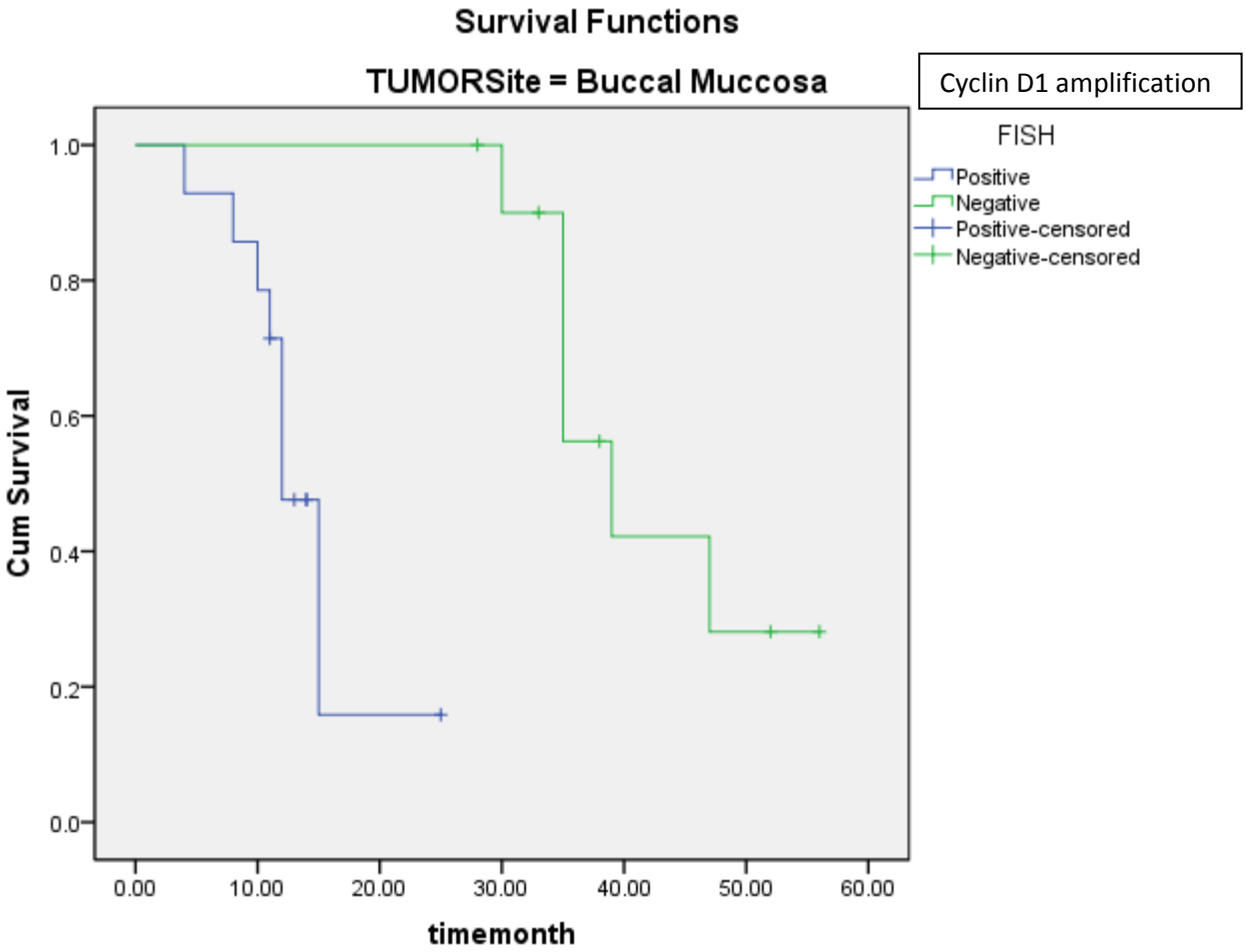

Figure 4.5 Survival rate patients with buccal mucosa cancer (n=25) in relation to Cyclin D1 amplification.