CHAPTER 4: RESULTS

4.1 Demographic characteristics

Twenty five male (55.6%) and 20 female (44.4%) subjects were included in this study. The age of the subjects ranged from 35 to 90 years with a mean of 59.0 years (standard deviation, 11.90) (Table 4.1). Twenty eight subjects (62.2%) were in their 5^{th} and 6^{th} decade of life at the time of diagnosis. Indian subjects made up the bulk of the study (68.9%) especially the female (95.0%) followed by the Chinese (22.2%) and Malay (8.9%) (Table 4.3).

Table 4.1. Distribution of cases according to age, gender and ethnic.

Demographic parameters	Mean (SD)	No, (%)
Age (years)	59.0 (11.90)	45 (100%)
Gender		
Male		25 (55.6%)
Female		20 (44.4%)
Ethnicity		
Malay		4 (8.9%)
Chinese		10 (22.2%)
Indian		31 (68.9%)

Table 4.2 showed detailed demographic characteristics, clinicopathological features and staining results for Ki-67, p53, MDM-2 and Bcl-2.

Table 4.2

Table4.2

Table 4.3 Demographic distribution according to gender and ethnic

Gender / Ethnic	Chinese	Indian	Malay	Total
Female	0 (0.0%)	19 (42.2%)	1 (2.2%)	20 (44.4%)
Male	10 (22.2%)	12 (26.7%)	3 (6.7%)	25 (55.6%)
Total	10 (22.2%)	31 (68.9%)	4 (8.9%)	45 (100.0%)

This study recorded 19 cases of buccal mucosa OSCC (42.2%), followed by 11 (24.4%) cases from the tongue. In addition, seven subjects (15.6%) had lesions on the palate while gum and lip recorded 2 (4.4%) and 3 cases (6.6%) respectively while the rest were found at the retromolar region (6.6%). A high number of buccal cases (18/45) were recorded in Indian subjects and most tongue SCC (7/47) was recorded in Chinese male subjects (Figure 4.1).

4.2 Clinicopathological features

A) TNM Staging with T and N Status

Current study could only record T, N and TNM staging for 35 cases. The distribution of cases according to T, N status and Stage is shown in Table 4.4 and 4.5. There was one at Stage I (2.86%), 10 at Stage II (28.6%), 9 at Stage III (25.7%) and 15 at Stage IV (42.84%).

Figure 4.1

B) Broders' Classification

Nineteen cases (42.2%) were graded as well differentiated OSCC in this study. Moderately differentiated OSCC was seen in 25 (55.6%) of the cases and one (2.2%) was poorly differentiated OSCC (Table 4.4).

C) Pattern of Invasion

This study recorded the following result for evaluation of pattern of invasion at the tumour advancing front (Table 4.4). Twenty percent of pattern of invasion type 1, 11.1% type 2, 55.6% type 3 and 13.3% type 4.

Applying Crissman et al. (1984) criteria for cohesiveness of the tumours whereby pattern 1 and 2 was considered as tumour exhibiting cohesiveness and 3 and 4 as non cohesive, this study recorded 68.9% of non cohesive tumours and 31.1% of cohesive tumours (Table 4.6). Furthur analysis revealed that 68.4% (13/19) well differentiated, 83.3% (15/18) moderately differentiated and 100.0% (1/1) poorly differentiated OSCC had non cohesive pattern of invasion (Table 4.6).

Table 4.4. Distribution of cases according to clinicopathological parameters

Clinicopathological parameters	No, (%)
TNM Staging (n=35)	
Stage I	1 (2.86%)
Stage II	10 (28.6%)
Stage III	9 (25.7%)
Stage IV	15 (48.84%)
Broders' Classification (n=45)	
Well differentiated	19 (42.2%)
Moderately differentiated	25 (55.6%)
Poorly differentiated	1 (2.2%)
Pattern of Invasion (n=45)	
Pattern 1	9 (20.0%)
Pattern 2	5 (11.1%)
Pattern 3	25 (55.6%)
Pattern 4	6 (13.3%)

Table 4.5 Distribution of cases according to T and N status

T/N Status	N0	N1	N3	Total
T1	1 (2.9%)	1 (2.9%)	1 (2.9%)	3 (8.6%)
T2	10 (28.6%)	2 (5.7%)	1 (2.9%)	13 (37.1%)
T3	2 (5.7%)	4 (11.4%)	2 (5.7%)	8 (22.9%)
T4	2 (5.7%)	3 (8.6%)	6 (17.1%)	11 (31.4%)
Total	15 (42.9%)	10 (28.6%)	10 (28.6%)	35 (100.0%)

Table 4.6 Distribution of Broders' Classification and pattern of invasion

Broders / Pattern of Invasion	Pattern 1	Pattern 2	Pattern 3	Pattern 4	Total
WD*	4 (8.9%)	3 (6.7%)	11 (24.4%)	1 (2.2%)	19 (42.2%)
MD	5 (11.1%)	2 (4.4%)	13 (28.9%)	2 (11.1%)	18 (55.6%)
PD	0 (0.0%)	0 (0.0%)	1 (2.2%)	0 (0.0%)	1 (2.2%)
Total	9 (20.0%)	5 (11.1%)	25 (55.6%)	3 (13.3%)	45 (100.0%)

*WD – well differentiated, MD – moderately differentiated, PD – poorly differentiated

4.3 Immunohistochemical expression

(A) Ki-67 Labeling Index (LI)

Ki-67 immunoreactivity was noted in 28 (62.2%) cases in this study (Table 4.7). The intensity of the staining ranged from very dark brown to faint and distributed in the nuclei at the basal layer of the tumour islands (progenitor compartment) and also the basal (progenitor compartment) and suprabasal (maturation compartment) layers of the tumour epithelium at the invasive front. The Labeling Index ranged from 15.9% to 70.2% with a mean of 23.4% (standard deviation, 22.85, median, 19.4%) (Figure 4.2 A)

(B) p53 expression

Thirty four subjects (75.6%) had overexpression of the p53 protein (Table 4.7). The overexpression was noted as dark brown nuclei staining distributed along the basal (progenitor compartment) and suprabasal (maturation compartment) layer at the tumour invasive front. They were also found in the nuclei at the basal layer of the tumours (progenitor compartment) and some in all cells within the tumour islands. The intensity of

the immunoreactivity varied within the subjects and between subjects from very dark homogenous brown to faint granulated in the nuclei. Six cases recorded the score of 1+ (13.3%), 8 cases with score 2+ (17.8%) and 46.7% (21/45) cases with score 3+. (Figure 4.2 B)

(C) MDM-2 expression

Strikingly, MDM-2 protein was detected in 44 of the subjects (97.8%) in this study (Table 4.7). A range of strong dark brown and pale brownish nuclei was noted in the tumours. Almost every nucleus in the tumours recorded the immunoreactivity and it was distributed along the basal (progenitor compartment), suprabasal (maturation compartment)layers at the tumour invasive front, including the keratinization layer and tumour islands with some diminishing immunoreactivity noted in the centre of the tumour islands in some cases. All cases recorded score 3+. (Figure 4.2 C)

(D) Bcl-2 expression

Brown cytoplasmic staining for Bcl-2 immunoreactivity was seen in two cases (4.4%) (Table 4.7). The immunoreactivity was confined to the basal (progenitor compartment) and suprabasal (maturation compartment) layers of the tumour islands at the tumour invasive front. Intensity ranged from strong dark brown to faint and was seen scattered around the nucleus in the cytoplasm in positive cases. Immunoreactivity was also seen in lymphocytes in cases with heavy lymphocytic infiltration. Both positive cases recorded score 3+ (4.4%). (Figure 4.2 D).

Figure 2.4

Table 4.7. Distribution of cases according to immunoreactivity of the markers

Immunoreactivity of markers		No, (%)
Ki-67 immunoreactivity		
	Positive	28 (62.2%)
	Negative	17 (37.8%)
p53 immunoreactivity		
	Positive	34 (75.6%)
	Negative	11 (24.4%)
MDM-2 immunoreactivity		
	Positive	44 (97.8%)
	Negative	1 (2.2%)
Bcl-2 immunoreactivity		
	Positive	2 (4.4%)
	Negative	43 (95.6%)

4.4 Relationship between Ki-67 status and Labeling Index (LI) and overexpression of p53, MDM-2 and Bcl-2.

No significant association was observed between the overexpression of p53, MDM-2 and Bcl-2 with Ki-67 status or its Labeling Index (LI). However, there were slight differences in the number of cases (approximately 20-40%) with positive Ki-67 status and overexpression of the proteins as compared to cases without overexpression (Table 4.8). Ki-67 LI was found to be higher in Bcl-2 oveexpressed cases (42.0%) than negative cases (17.8%), slightly higher in MDM-2 overexpression (20.0%) and no Ki-67 immunoreactivity in the only one negative case and slightly low in p53 overexpression (17.6%) than cases not expressing p53 protein (22.05%) (lower or higher than overall median Ki-67 LI, 19.4%) (Table 4.9).

Table 4.8. Relationship of immunoreacitivity of markers to Ki-67 status

Markers	No (n)	Ki-67 status		*p* value[a]
		Positive, n (%)	Negative, n (%)	
p53 immunoreactivity				
Positive	34	20 (58.8%)	14 (41.2%)	0.719
Negative	11	8 (72.7%)	3 (27.3%)	
MDM-2 immunoreactivity				
Positive	44	28 (63.6%)	16 (36.4%)	0.378
Negative	1	0 (0.0%)	1 (100.0%)	
Bcl-2 immunoreactivity				
Positive	2	2 (100.0%)	0 (0.0%)	0.519
Negative	43	26 (60.5%)	17 (39.5%)	

[a] Fisher's exact test. Significant at $p<0.05$

Table 4.9. Relationship of immunoreactivity of markers to Ki-67 Labeling Index (LI)

Markers	No (n)	Ki-67 LI	Z Statistic[b]	*p* value[b]
		Median (IQR)		
p53 immunoreactivity				
Positive	34	17.6 (43.5)	-0.489	0.556
Negative	11	22.05 (47.9)		
MDM-2 immunoreactivity				
Positive	44	20.0 (43.2)	-1.108	0.268
Negative	1	- (-)		
Bcl-2 immunoreactivity				
Positive	2	42.0 (-)	-1.132	0.257
Negative	43	17.8 (42.4)		

[b] Mann-Whitney test. Significant at $p<0.05$

4.5 Relationship between Ki-67 status or Labeling Index (LI) and overexpression of p53/Bcl-2, p53/MDM-2 and MDM-2/Bcl-2

No significant association was again noted when comparing the status of Ki-67 or the Labeling Index (LI) with the combined overexpression of p53, MDM-2 and Bcl-2. However, there is a marked difference in the number of cases overexpressing Ki-67 (approximately 40%) that simultaneously overexpresses both MDM-2/Bcl-2 and p53/Bcl-2 as compared to cases which recorded the absence of either one or both of the proteins. (Table 4.10). Comparing the Ki-67 LI among combined overxpression of the markers to median overall Ki-67 LI (19.4%) shows that higher Ki-67 LI was found in cases overexpressing MDM-2/Bcl-2 and p53/Bcl-2 (42.0%) and slightly higher in those overexpressing p53/MDM-2 (Table 4.11).

Table 4.10. Relationship of combined expression of markers to Ki-67 status

Markers	**No (n)**	**Ki-67 status**		***p* value**[a]
		Positive, n (%)	**Negative, n (%)**	
p53/MDM-2 immunoreactivity				
Positive	34	21 (61.8%)	13 (38.2%)	1.000
Negative	11	7 (63.6%)	4 (36.4%)	
MDM-2/Bcl-2 immunoreactivity				
Positive	2	2 (100.0%)	0 (0.0%)	0.519
Negative	43	26 (60.5%)	17 (39.5%)	
p53/Bcl-2 immunoreactivity				
Positive	1	1 (100.0%)	0 (0.0%)	1.000
Negative	44	27 (61.4%)	17 (38.6%)	

[a] Fisher's exact test. Significant at $p<0.05$

Table 4.11. Relationship of combined expression of markers to Ki-67 Labeling Index (LI)

Markers	No (n)	Ki-67 LI	Z Statistic[b]	*p* value[b]
		Median (IQR)		
p53/MDM-2 immunoreactivity				
Positive	34	18.5 (44.0)	-0.190	0.849
Negative	11	21.6 (42.4)		
MDM-2/Bcl-2 immunoreactivity				
Positive	2	42.0 (-)	-1.132	0.257
Negative	43	17.8 (42.8)		
p53/Bcl-2 immunoreactivity				
Positive	1	42.0 (-)	0.000	1.000
Negative	44	17.8 (42.4)		

[b] Mann-Whitney test. Significant at $p<0.05$

4.6 Relationship of demographic and clinicopathological parameters with Ki-67 status, overexpression of p53, MDM-2 and Bcl-2

Table 4.12 showed relationship between demographic and clinicopathological parameters with Ki-67 status, overexpression of p53 and Bcl-2. Although no significance was noted in any categories, slight differences (approximately 20%) were noted in the number of cases for overexpression of Ki-67, p53 and MDM-2 in the Age category, p53 in Gender and Ethnic and Bcl-2 in Site as compared to those without.

4.7 Relationship of demographic and clinicopathological parameters with combined expression of the markers.

Table 4.13 showed relationship between demographic and clinicopathological parameters

with combined expression of the markers investigated. None of the combined expression of the markers was found to be associated with the parameters but there was an approximate 20% different in the number of cases overexpressing Ki-67/p53, Ki-67/MDM-2 and p53/MDM-2 in the Age category and Ki-67/p53 in Ethnic as compared to those without coexpression.

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Table12

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Table13

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Table 13

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