

CHAPTER 5

DISCUSSION

In this study, we adopted FISH technique to detect cyclin D1 deregulation. The incidence of positive cyclin D1 amplification in overall cases was 36 cases (72%) which is similar to that in previous reports, Callender et al. (1994) and Kyomoto et al. (1997).

5.1. Sociodemographic characteristic:

The sample size of this study is relatively small as compared to other studies reported in the literature. Choi et al. (2006). RF de Araújo Júnior et al. (2008). In the current study, 50 cases were selected from year 2004 until 2010. Costello and Osborne (2005) tested the effect of sample size on the results of factor analysis reporting that larger samples tend to produce more accurate solutions.

5.1.1. Age:

The mean age in the current study was 60 years, in which 80% of the patients were in the 5th- 9th decade of life. This result concurs with previous studies that reported OSCC as a disease of older age groups in Malaysia (Ramanathan & Lakshimi 1976, Ng et al. 1985; Siar et al. 1990 and Ng & Siar 1997). While Burzynski et al. 1992 reported the incidence of oral cancer in patients less than 40 years of age about 3%. In the present study, there were three cases of young patients below 40 years old.

The present study showed that association of cyclin D1 amplification statistically insignificant with age for both tongue and buccal mucosa SCC. There is unequal distribution of age groups for tongue, therefore, the association between CD1 amplification and age cannot be confidently concluded separately for each group. However, if considered as a whole group, age cannot be positively correlated with CD1 amplification. (i.e. we cannot say that the older the patient, the higher CD1 amplification).

5.1.2. Gender:

In the present study there are more female than male with a female to male ratio of 1.7:1. Previous local studies reported by Ng et al. (1985) and Siar et al. (1990) reported an overall female preponderance although the F:M ratio was smaller 1.1:1 and 1.5:1 respectively. Data from local studies have revealed that frequency of certain anatomical site involvement of OSCC vary with gender distribution (Ramanathan & Lakshimi 1976; Ng et al.1985). Generally, female preponderance was noticed in SCC of the buccal mucosa while male preponderance was found in SCC of the tongue. Thus, the distinct difference in the F: M ratio observed may be attributed to habits that predisposed to OSCC, as the present study showed. No significant statistical association between cyclin D1 amplification with gender.

5.1.3. Ethnicity:

Comparison among the racial differences of OSCC in previous studies showed that Indians were the predominant group (Ramanathan & Lakshimi 1976; Ng et al.1985, Siar et al. 1990). This distinct pattern of racial distribution of OSCC was also observed in the current study. Whereby, the Indian ethnic group represents 68% of total study sample.

Studies showed that the frequency of anatomical site involvement in OSCC also varies with the ethnic group (Ramanathan & Lakshimi 1976; Ng et al.1985). The dominant site was found to be the buccal mucosa among Indians while tongue was the most frequent site for Chinese samples (Ramanathan & Lakshimi 1976; Ng et al.1985, Siar et al. 1990). For the Malays, studies showed that tongue and buccal mucosa were equally dominant as reported by Ramanathan & Lakshimi (1976). In the current study, the dominant sites for both Indian and Malays were buccal mucosa 65%, 70% respectively while for Chinese the tongue showed 84%. However, in the present study, amplification of cyclin D1 has no significant statistical association with ethnicity.

5.2. Clinicopathological characteristic:

5.2.1. Tumor site:

Study sample consist of equal number of cases for both tongue and buccal mucosa. The positive amplification of cyclin D1 was recorded in 88% of tongue SCC cases and 54% of buccal mucosa SCC. A study by Fujii et al. (2001) coincided our result by recording positive amplification of cyclin D1 in 56.5% of tongue SCC.

Tongue and buccal mucosa SCC represent a different biological subentities, etiology as well as genetics and these differences should be considered in the management of OSCC (Kannan et al. 1997). There are differences in the effectiveness of the molecular pathway which participate in the development of tongue and buccal mucosa SCC (Sathyan et al. 2006).

The result of this study showed a significant difference between the amplification of cyclin D1 in tongue and buccal mucosa SCC (p -value=0.012), and this suggests that may be differences in the behavior of squamous cell carcinoma in tongue and buccal mucosa.

5.2.2. Tumor depth:

In the present study, the cut-off point of 5mm was used as various studies had considered tumor depth as a predictive factor for nodal metastasis and survival rate (Al-Rajhi et al. 2000, Gonzalez-Moles et al. 2002 and O-charoenrat et al. (2003). Okamoto et al. (2002) and Myo et al. (2005) found that tumor depth more than 4mm were significantly predictive for Cyclin D1 positively amplified.

This study revealed that 92% of tongue SCC cases and 96% of buccal mucosa SCC have tumor depth of 5 mm or more. This findings are in accordance with the findings of other studies that showed higher percentage of OSCC cases with tumor depth more than or equal to 5mm. (Gonzalez-Moles 2002, Kane et al. 2006).

For tongue SCC, there was a significant statistical association between the amplification of cyclin D1 and tumor depth of 5mm or more. Cyclin D1 positivity was identified in 22 (95.7%) cases of tongue SCC with tumor depth more than or equal to 5 mm, while none were identified with tumor depth less than 5 mm. This result is similar to that reported by Wang et al. (2006). There is no significant association between amplification of cyclin D1 and tumor depth in buccal mucosa cases.

5.2.3. Pattern of invasion:

Analyzing the pattern of invasion, the current study noted that the majority of cases (88%) were non-cohesive type. The same trend was observed in a western study by Spiro et al. (1999) who found that 61% of OSCC patients had non-cohesive pattern of invasion. Other studies have also reported results showing higher percentage of OSCC with non-cohesive type of pattern of invasion (Miyamoto et al. 2003; Kane et al. 2006; Boey 2002; Abdul Jalil 2003).

The current study showed no significant difference between pattern of invasion and cyclin D1 amplification for both tongue and buccal mucosa SCC. Shiraki et al. (2005) had also reported no significant association between overexpression of cyclin D1 and pattern of invasion in OSCC

A study conducted by Myo et al. (2005) showed that cyclin D1 numerical aberrations is always associated with overexpression of cyclin D1 protein. Thus, overexpression of this protein may affect the phenotypes of cancer cells. Cyclin D1 encodes an important regulatory protein that promotes cell cycle progression by activating CDK 4 and 6. However, recent studies have indicated that cyclin D1 affects the activity of various non-CDK-dependent cellular transcription factors such as estrogen, DMP1, STAT3 and BETA2/NeuroD (Lamb 2003).

It is not clear how overexpression or amplification of cyclin D1 protein affects the invasive and metastatic behavior of cancer cells, thus, its CDK-independent biological activities that may contribute to the acquisition of the invasive ability and metastatic potential of tumor cells.

However, overexpression of other oncogenes on the 11q13 amplicon, such as *EMSI* and *TAOSI*, may also contribute to the development of invasive and metastatic characteristics of cancer cells (Huang et al. 2002). Therefore, this issue is still a matter of controversy and further investigations are required.

5.2.4. pT:

In a study conducted by Chheitri et al. (2000), 59% of OSCC cases had tumor size of T1 and T2 and 41% had tumor size of T3 and T4. Similarly, Kuo et al. (1999) and Shraki et al. (2005) reported higher percentage of T1 and T2 compared to T3 and T4.

The current study had more OSCC with T1 and T2 tumor size (66%) as compared to T3 and T4 (34%). Nevertheless, tumor size has not been particularly effective in predicting the metastasis and survival rate as reported by Gonzales-Moles et al. (2001), Kane et al. (2006) and Woolgar (2006).

The present study showed significant statistical difference between cyclin D1 positivity amplification and tumor greatest dimension for tongue SCC cases, while for buccal mucosa SCC cases there is no significant difference statistically. This finding was completely opposite with a study done by Fujii (2001) in which cyclin D1 amplification was not significant with tumor size in tongue SCC.

5.2.5. pN:

The present study showed that (44%) of tongue SCC and (64%) of buccal mucosa SCC had positive lymph node. This findings is in contrast with what was reported by Woolgar (2006) that nodal metastasis were diagnosed histologically in 59-64% of SCC of the tongue compared to 22% of buccal mucosa SCC.

Nevertheless, the anatomical site is not the sole factor affecting the distribution of metastatic lymph nodes, as socioeconomic constraint such as delay in diagnosis and seeking treatment may affect the possibility of metastasis. A study in India done by Ghoshal et al. (2006) involving only the buccal mucosa revealed that 62% patients with OSCC had lymph node metastasis. Possible that more buccal mucosa cancer patients present late.

The current study showed that amplification of cyclin D1 was seen in 90.9% and 68.8% for tongue and buccal mucosa cases respectively with lymph node metastasis, and 85.8% and 33.3% for tongue and buccal mucosa cases respectively with non-metastatic lymph nodes. Thus, this association was statistically not significant which leads to the inability to reject the null hypothesis.

Fortin et al. (1997) did not observe any correlation between lymph node status and cyclin D1 amplification in patients with oral and oropharyngeal carcinomas. Fujii (2001) also found there was no correlation between cyclin D1 amplification and lymph node involvement in tongue SCC.

The studies conducted by Miyamoto R et al. (2002) and Myo K et al. (2005) on FISH technique utilized fresh fine needle aspirated biopsies and that might be the reason for the significant results obtained (Cyclin D1 numerical aberration were observed significantly associated with the presence of lymph node metastasis), whereas this study used paraffin-embedded tissue which was thought to be less feasible.

5.2.6. pTNM staging:

There are no reports found on pTNM stage of OSCC patients in Malaysia. Most studies in Malaysia involved clinical stage TNM as one of the parameters (Boey 2002, Abdul Jalil 2003, Lee 2006). This could be due to limitation of surgically resected cases at the diagnostic laboratory in previous years. The current study on surgically resected tumors revealed that the majority of the cases (64%) were pathological stages III and IV and the remaining cases (36%) were stages I and II. This finding is similar to a recent study done on Indian the population by Ghoshal et al. (2006), which showed that only 20% OSCC patients were in stages I and II and the remaining majority of 80% were in stages III and IV disease.

The current study showed that amplification of cyclin D1 has no significant statistical association with pTNM stage. The present finding is in contrast with a study done by de Vincente et al. (2002) which showed that overexpression of cyclin D1 was found to be positively correlated with advanced pathological tumor stage of SCC in oral cavity.

Michalides et al. (1995) and Bartkova et al. (1995) reported no association of cyclin D1 amplification with tumor stage in OSCC which supports the finding of the current study. However, Fujii et al. (2001) who studied the amplification of cyclin D1 only in tongue SCC reported that there is no correlation between the amplification of cyclin D1 and the associated tumor stage. More recent studies done

by Kuo et al. (2002) and Wang et al. (2006) reported that there is no correlation between positive expressions of cyclin D1 with clinical stage in OSCC.

5.2.7. Modified Broder's grading:

The present study revealed that the most frequently encountered type of OSCC according to modified Broder's grading was moderately differentiated SCC. Only 16% of the samples were poorly differentiated, all of which were from tongue SCC.

In different studies in Malaysia, the well-differentiated type was seen more commonly than the moderately differentiated type while the current study showed the highest prevalence being the moderately differentiated type. Siar et al. (1990) reported that well differentiated OSCC in 81.1% of the cases, 17.9% moderately differentiated and only 1% of poorly differentiated cases. Boey (2002) reported that most 51.9% and 33.3% of his study samples were well differentiated and moderately differentiated respectively and the remaining 14.8% of were poorly differentiated.

The current study coincides with most studies that reported a minority of OSCC patients with poorly differentiated type (Chhetri et al. 2000, Amaral et al. 2004, Rodolica et al. 2005). Kane et al. (2006) also reported higher percentage of moderately differentiated OSCC cases compared to poorly differentiated.

This study showed that there was no statistical significant association between cyclin D1 amplification and tumor grading. This is consistent with other studies done by

Kuo et al. 1999, Nakahara et al. (2000), de Vincente et al. (2002) and Wang et al. (2006) which stated that overexpression of cyclin D1 was not associated with histological grading in OSCC. However, Bova et al. (1999), Miyamoto et al. (2002) and Angadi & Lrishnapallai (2007) found that an amplification of cyclin D1 was associated with increased tumor grade in OSCC.

5.3. Survival rate:

In the present study, Kaplan-Meier survival analysis (KMSA) was performed and for cyclin D1 negative patient recorded a survival rate of 57.1%. This is considerably lower when compared to the study of Fujii et al. (2001) where the 5-year disease-free survival rate was 80.0% for negative patients, a result which was significantly better for cyclin D1 amplification negative patients. The reason for the difference in the result is that Fujii et al. (2001) studied only the tongue SCC whereas in the present study both tongue and buccal mucosa SCC were included and besides that, this study considered over-all survival rate instead of disease-free survival rate due to lack of information with regards cause of death. However, the Log Rank (Mantel-Cox) test for the current study resulted in a significant association between survival rate and cyclin D1 positive and cyclin D1 negative overall patients ($p=0.009$) and significant association between survival rate and cyclin D1 positive and cyclin D1 negative in buccal mucosa patients ($p<0.001$).

The KMSA analysis based on tumor site was performed where the survival rate was 60% for tongue SCC and 40% for buccal mucosa SCC patients. The Log Rank (Mantel-Cox) test did not show any significant association between survival rate and cyclin D1 amplification for tongue SCC. This was in contrast to Sathyan et al. (2006) who studied the overexpression of cyclin D1 in tongue SCC. However, there was significant result observed between survival rate and cyclin D1 amplification in buccal mucosa SCC which was similar to Liu et al. (2004) who studied the overexpression of cyclin D1 in buccal SCC.

5.4. Limitation of the study:

The main setback of this study was the limited size of the sample. We were constrained by the availability of surgical specimens in our centre (Oral Pathology diagnostic archive at the Faculty of Dentistry, University of Malaya). Time constraint did not allow us to seek joint collaboration with other centres to increase the sample size.

CHAPTER 6

CONCLUSION, IMPLICATION and RECOMMENDATIONS

6.1. Conclusion:

In summary, the results of this study demonstrated that:

1. The amplification of cyclin D1 was identified in 72% (36) of total OSCC cases and the majority were tongue SCC (22).
2. Amplification of cyclin D1 in tongue SCC cases was significantly associated with only tumor depth (≥ 5 mm) and tumor greatest dimension.
3. No significant association between amplification of cyclin D1 in buccal mucosa SCC with any of the sociodemographic and clinicopathological parameters.
4. Survival rate was only significantly better for cyclin D1 negative amplification of buccal mucosa SCC.

6.2. Implication of the study

These results indicate that cyclin D1 has a potential to be recognized as one of the promising prognostic markers in cancer. If cyclin D1 can be proven as equally reliable as other histopathological prognostic indicators, then a serum assay of cyclin D1 has a potential to be of great value for the surgeons to set a better and more accurate plans. Therefore, further studies with larger sample size are strongly recommended in order to confirm the significance of cyclin D1 as a prognostic marker.

6.3. Recommendation

Based on the current study, it is recommended that:

- Larger sample size should be studied to validate the potential of cyclin D1 as the prognostic factor as it would give a more definitive result on the relationship of cyclin D1 amplification with the sociodemographic, clinicopathological parameters.
- Patients with good record data are included in the future studies as this study suffers from the lack of data on the cause of death and status of patients until last follow up.
- CDK independent pathway have to be investigated.
- Further wider studies to evaluate FISH technique as a routine investigation technique with estimating the cost value.