Chapter-5: Discussion

5.1 Inference of epidemiological, lifestyle and biochemical factors on ESCC

Esophageal squamous cell carcinoma in the Asian region is the most common cancer among the different histological types of esophageal cancer which in certain region may accounts for 95% (Higuchi, Koizumi et al. 2009). In this study, male ESCC patients were reported approximately two times more than their female counterpart with a male to female ratio of 2:1. This is similar to literature report of male to female ratio of 1.8:1 (Mohiuddin et al., 2013). Moreover, female ESCC patients tended to live longer than their male counterpart in our findings. The median age was reported 55 years (range 33-77 years) at the time of ESCC diagnosis in our study which is lower in comparison to reported median age of 77 years (range 73-81 years) in a Japanese study (Ohba, Kato et al. 2016). It indicates that incidence of ESCC is occurring in young age group in the northeast India.

82% of the patients in the present study reported good initial performance status (PS 0 or 1), which is an indicator of good general health which correlates with the similar finding of 88% performance status among patients (Chen, Xu et al. 2014). Good performance statuses of the patients can impacts on their better survival since they can tolerate the cancer directed therapy well than patients with poor performance status.

Difficulty in swallowing (Dysphagia) found in predominantly 96% of the ESCC patients in this study whereas it was reported 92% (Saddoughi, Taswell et al. 2016), 54% (Ripley, Sarkaria et al. 2016), 77% (Okello, Churchill et al. 2016) pointing that dysphagia is the most common symptom for esophageal cancer including rare pathological types diagnosis. In our group of ESCC patients, we found nearly 99% of the esopaheal cancer

started in the thoracic region of the esophagus which correlates with the usual finding that 90% of the esophageal cancer starts in the thoracic region (Higuchi, Koizumi et al. 2009).

We reported 77.7% of the ESCC patients using tobacco either through smoking or in smokeless form whereas 28.7% drink alcohol and 81.5% chew areca nut. The role of tobacco and areca nut chewing, tobacco smoking and alcohol drinking have shown to be a risk factor in the tumorogenesis of different types of squamous cell carcinoma including ESCC (Lee et al., 2005; Ko et al., 1995; Muwonge et al., 2008; Lagergren and Lagergren 2010; Pramesh et al., 2015).

5.2 Molecular markers ALDH1, HER2 & p16 impact on treatment response and prognosis in ESCC

Cancer stem cells are now widely considered as the reasons behind the maintenance of tumor growth and conventional treatment failure (Hu and Fu 2012, Vidal, Rodriguez-Bravo et al. 2014). In this study, we found ALDH1 positivity in 65.7% of ESCC patient. Whereas Minato group reported ALDH1 expression in 25% of ESCC patients who availed NACT and mean expression of 39.5% in all ESCC studied patients irrespective of NACT administration (Minato, Yamamoto et al. 2013). ALDH1 expression was found significantly associated with poor response after NACT among ESCC patients in our study (P<0.001). However, this did not correlate with the finding of Minato et al (2013) where they reported no such association, which could predict the response to neo adjuvant chemotherapy though they concluded that ALDH1 acts as a predictive marker for poor prognosis in ESCC.

Wang et al (2012) reported that there was a significant decrease in the 5 year overall survival of the patients with ALDH1 positive tumor than those with ALDH1

negative tumor (Wang, Zhe et al. 2012). We however couldn't found any correlation between ALDH1 positivity and survival. Though there were significant deaths among poor responders in this study (*P*=0.009). This observation remarks the impact of treatment failure on the survival of the patients irrespective of their ALDH1 or HER2 positivity status.

In this study, 7.4% ESCC patients were found to be HER2 positive. This correlates with the finding of HER2 positivity in 6.5% studied patients (Sato-Kuwabara, Neves et al. 2009). Another study where HER2 gene amplification was assessed in ESCC, 3.9% patients were found to be HER2 positive and it correlated with tumor and vascular invasiveness with lymph node metastasis (Huang, *Zhao* et al. 2013).

We couldn't observed any correlation between HER2 positive status with either treatment response (*P*=0.56) that seems to fit well with the finding of Huang et al (2013). Although, in a contrasting report, it was reported that In the systemic chemotherapy group, HER2 along with EGFR, and HER3 correlated with therapeutic response (Yamamoto, Yamai et al. 2012).

A significant correlation was seen between patients with high ALDH1 and HER2 expression (P=0.034) in this study. Similar findings of association of ALDH1 and HER2 expression (P= 0.006) were reported (Bi, et al. 2012). In metastatic breast carcinoma ALDH1 correlated to negative estrogen receptor and positive HER2 status in circulating tumor cells (Gradilone, Naso et al. 2011). Ginestier also reported similar findings (Ginestier, Hur et al. 2007). This may be aiding the therapy resistance cascade and high tumor proliferation due to overexpression of resistance conferring genes like YAP1, a feature that was usually found in ALDH1 over expressing cells (Ajani, Wang et al.

2014). These proved as interesting therapeutic targets. Verteporfin a small molecule inhibitor can block cancer stem cell properties in YAP1 and ALDH1 positive cells (Song, Ajani et al. 2014).

Our understanding of the role of HPV in carcinogenesis is evolving. It is strongly implicated as a causative factor in the tumorogenesis of squamous cell carcinoma of cervix (Walboomers et al., 1999; Hausen, 2009). There is a strong association of HPV with squamous cell cancer of the head and neck, commonly oropharynx (D'Souza et al., 2007), where p16 expression act as predictive marker for response to radiation therapy. Its role in esophageal squamous cell carcinoma (ESCC) (Liyanage et al., 2013) is unclear. However, it is implicated in some studies as predictive and prognostic marker of squamous cell carcinomas (Lassen et al., 2009; Fischer et al., 2010; Cao et al., 2014).

p16 has been considered surrogate marker for HPV (El-Naggar and Westra 2012). It's over expression in ESCC was found in 22% (24 out of 108) of studied cases in our study. It correlates with the results of a recent meta analysis of 13832 ESCC patients involving 124 studies, where the average prevalence of HPV was found 0.304 (0.185, 0.423) by immunohistochemistry with 95% confidence interval (Petrick et al., 2014). A similar meta analysis based on 132 studies related to HPV in ESCC by H. A. Hardefeldt found HPV prevalence in ESCC at 24.8% (H. A. Hardefeldt et al., 2014).

However there exists inconclusive evidence of its aetiological significance in the development of ESCC (Mohiuddin et al., 2013; Zhang et al., 2014). Few Meta studies though have reported that HPV infection can increase the risk of developing ESCC by 3 folds (Liyanage et al., 2013).

p16 expression was however more common in ESCC in women's. We reported 26% of ESCC in females and 21% in males over expressed p16 (P=0.54) in this study.

This study reported 69% ESCC tumors were in moderately differentiated grade where 58% were shown p16 positivity. In squamous cell carcinoma of cervix, p16 positivity has been reported in 100% of patients (Ma et al., 2010). We found p16 expression was more prevalent in mid thoracic region (54%) than upper (29%) and lower thoracic region (17%) that may be due to its proximity to oropharyngeal squamous epithelium, which acts as route for HPV infection. Similar result was also reported by Fangli et al., 2014.

We found 50% those with pathologically complete response were p16 positive on the contrary only 15% non responders were positive for p16. In this study we have found more number of responders than non responders with the expression p16 (P<0.001), which indicates that p16 positive tumors show favourable outcome in terms of reaching pathologically complete response (PCR) of tumor after the complete regime of neo adjuvant chemotherapy. Patients who showed p16 positivity were reported 42% death among all reported deaths (P=0.097). This correlates with similar finding where HPV positivity was shown to increase progression free survival and disease free survival in patients with squamous cell carcinomas (Ang et al., 2010; Fischer et al., 2010; Fangli et al., 2014).

Current data though suggests the role of p16 as predictive marker for treatment response assessment however a similar study on a larger number of ESCC patients will provide further evidence that is required to implement this finding in routine clinical settings for better management of ESCC.

5.3 Impact of cell cycle parameter on clinical course of ESCC

One of the most widespread use of flow cytometry has been to study the cell cycle kinetics through DNA ploidy and proliferative index (S phase fraction) estimation in a wide range of biological specimens both plants and animals. Employing the flow cytometer based cell cycle analysis in differnt tumor type helps in the understanding of its total DNA content and how rapidly cells are dividing through ploidy and S phase fraction estimation respectively and their impact on the prognosis (Merkel, Dressler et al. 1987, Xue, Wu et al. 2016). It gives us an overall picture of chromosal instability that is one of the hallmarks of cancer. Flow cytometer based cell cycle analysis has been used in esophageal squamous cell carcinoma (Chanvitan, Puttawibul et al. 1997). In the current study, cell cycle analysis was performed on 108 esophageal squamous cell cancer specimens to determine the S phase fraction and DNA ploidy status. Aneuplody was reported in 94% of the studied ESCC tumors that nearly coorelates with the findings of 84% aneuploidy in ESCC reported by (Chanvitan, Puttawibul et al. 1997). Responders reported in this study were having high S phase fraction (P=0.041) which makes sense since chemotherapy works on fast dividing cancer cells.

5.4 A proposed model for treatment response assessment in ESCC

A model for treatment response assessment for patients with esophageal squamous cell carcinoma could be proposed based on the results of this study.

NACT Response Assessment	Tumor status	Node status	ALDH1 expression	P16 expression	Ploidy status	S phase fraction	Initial ECOG PS
Responder	Low grade	Negative	Negative	Positive	Diploid	High	Good
Non responder	High grade	Positive	Positive	Negative	Aneuploid	Low	Poor

Table 40. A proposed model for response assessment against neo adjuvant chemotherapy for esophageal squamous cell carcinoma patients.

According to this proposed model as described in table 40, those ESCC patients who will respond to NACT will report low grade tumor, negative node status, negative ALDH1 expression, positive p16 expression, good performance status (PS), diploid tumor with high S phase fraction. Whereas those who will report high grade tumor, node positivity, ALDH1 over-expression, no p16 expression, poor initial performance status, aneuploid tumor with low S phase fraction possibly not respond to neo adjuvant chemotherapy in esophageal squamous cell carcinoma.

Life style, biochemical and epidemiological factors were found in this study of limited importance in context of predicting the clinical outcome in ESCC. Although the prevalence of areca nut (81%), pan (79%), cigarette (55%), tobacco chewing (47%) was high in ESCC patients, we found no statistical significance of these factors with p16 expression. Extensive use of areca nut and tobacco in this region corresponds to the finding of highest esophageal cancer AAR of 71.4 in males and 30.2 in females reported in East Khasi Hills of Meghalaya state in north east India whereas in Asia it reported second highest in China, Jiashan at AAR 20.2 for males and in Pakistan, South Karachi at AAR 8.6 for females (NCRP 2013).

The percentage of alcohol use among ESCC patients was found considerable low at 29% in our study. It shows no correlation with the available report where alcohol was considered as one of the important risk factor for developing ESCC (Blot, 1999; Lagergren and Lagergren 2010).