Chapter 5

Discussion

5.1 Distribution of socio-demographic profile

In this case-control study, the difference in the mean age was highly significant between the cases and controls (p = 0.000). The mean age of cases was 59.6 years, almost 20 years older as compared to the controls, whereby the mean age was 40.8 years. These patients were recruited from OCRCC database that involved patients from various age groups who attended nine selected centers for minor ailments or cancer problems. Since in this study, the age of the study subjects was not matched, perhaps the difference in the mean age between cases and control was also reflecting the actual scenario in the population where older people were more prone to suffer from oral cancer. The present findings also concur with previous findings that oral cancer in Malaysia is a disease of the older age group where majority of the patients were in the fifth to seventh decade of life (Ramanathan and Lakshimi, 1976; Ng and Siar, 1992).

With regards to gender distribution, significant difference was found in the distribution of males and females among the cases and controls (p = 0.039). In lung cancer studies, one study done in United States by Wang *et al*., (2004) also showed significant difference in gender distribution between cases and the controls, but Spitz *et al*., (2000) on the contrary showed no statistical significant between these two genders. Thus, it suggests that many factors could play the role in contributing to the difference in gender distribution among cancer patients. These may include geographical area, genetic susceptibilities, lifestyles and also which gender is more exposed to the high risk habit in a particular population. In our study for example, though the distribution of males and females was similar in control group, female represented nearly two third of

cases. One way to explain this finding was while it is still dependent on cancer types; on the whole females were at the higher risk of getting cancers and perhaps this is true for oral cancer because more females practice higher risk habit such as chewing betel quid (Zain, 2001).

The ethnic distribution of Malays, Chinese, Indians and the Indigenous was highly significant among cases and controls ($p = 0.000$). The racial composition of the cases showed that the Indian were the predominant group (42.6%). This was followed by the Indigenous (24.3%), Malays (19.1%) and only 13.9% were Chinese. This pattern of ethnic distribution in cases in this study was almost similar to the incidence report of mouth and tongue cancers in the general population where the majority of mouth and tongue cancers cases were the Indians with 47.9%, followed by the Malays and the Chinese with 26.2% and 26.0% respectively. In this 2003 NCR second report, however, the ethnic distribution does not encompass the Indigenous group (Lim and Halimah, 2004). On the other hand, in our study, the slight difference noted in the incidence rates between Malay and Chinese, possibly was attributed to the difference in our data collection which also involved East Malaysia. At the same time, Ramanathan and Lakshimi (1976) previously reported that the prevalence rate of oral cancer is low among the Malays and Chinese but high (60%) among the Indians when compared to the local population ratio. This distinct pattern of racial distribution of oral cancer was also observed in the current study.

Exposure to alcohol drinking was reportedly to be highly significant among cases and controls ($p = 0.000$). More than ninety percent of the control subjects were not associated with drinking habit. In this study, only one-third among the cases consumed alcohol as compared to 65.9% and 56.6% of drinkers among the cases in

Taiwan and Southern Thailand studies, respectively (Hung *et al*., 1997; Kietthubthew *et al*., 2001). This perhaps is due to the fact that drinking alcohol is prohibited among Malays.

A significant number of subjects with betel-quid chewing habit were present in this study (p = 0.000). More than half (55.7%) of the cases were betel-quid chewers while 93.1% of the controls were non betel-quid chewers. These findings were similar to a Taiwanese study by Hung *et al*., (1997) which reported the proportion of betel-quid chewers were 73.2% among the cases while among the controls 87.8% were non betel-quid chewers. The high number of subjects in cases who chewed betel-quid are most likely consisting of the Indians and the Indigenous people of Sabah and Sarawak. This explanation is supported by Zain *et al*., (1997) which in her study found that these ethnic groups (Indians and the Indigenous people of Sabah and Sarawak) were indulging on betel-quid chewing habit regularly.

Among the risk habits examined in this study, tobacco smoking was found to be not statistically significant different between cases and controls (p = 0.610). The proportion of current and former smokers was higher among the controls (35.3%) than the cases (32.2%). Interestingly, more than two-thirds of the subjects in cases were never smokers. Except for a study in Southern Thailand (Kietthubthew *et al*., 2001), most studies shown that the proportion of current and former smokers were usually higher among the cases (Hung et al., 1997; Olshan *et al*., 2000; Cha *et al*., 2007). One suggestion is for future studies to look into the role of smoking-alcohol interaction and smoking-betel-quid interaction in oral cancer development because a possible reason for the similar proportion of never smoker and smoker in cases and control is that smoking alone does not contribute to having oral cancer.

Another factor which is also important to be considered and perhaps has some influence on the proportion of cases and controls being alcoholic, betel quid chewer and smoker was how patients actually interpreted and classified themselves. Social drinkers and betel quid chewers may consider themselves as non drinker and non betel quid chewer whereas looking into smoking habit should actually take into account the duration and amount of cigarettes they smoke. However, this was not captured in our study since there was limitation in the secondary data used, thus future studies should not overlooked on this matter.

There was no significant difference on the family history of cancer among the cases and controls ($p = 0.389$). Although it was not statistically significant, the proportion of cases with family history of cancer is higher than the controls. This observation holds true for other lung cancer studies (Chan-Yeung *et al*., 2004; Cote *et al*., 2005). However, it will be more interesting to really discover how true family history can influence the risk of having oral cancer, thus future studies should be done to investigate on this. Discovery would help in identifying high risk group of developing oral cancer.

5.2 Distribution of dietary ITC intake and GSTs polymorphisms

In this study, the dietary ITC intake per 1000kcal was recorded as the continuous data. The distribution of estimated intake level of ITC was skewed to the right. Unfortunately, till date there is no standard cut off point introduced to accurately determine the measurement of dietary ITC intake, and hence the median value of the

distribution was chosen as the cut off point to dichotomize the dietary ITC intake into a meaningful high and low ITC intake. Median value was a better measurement of central tendency to describe the skewed distribution in this study as compared to other descriptive statistics such as mean, tertiles or quartiles since median was the least affected by the extreme values of dietary ITC intake. Some other studies also used the median as the cut off point (Spitz *et al*., 2000; Zhao *et al*., 2001; Seow *et al*., 2002) while others utilized the tertiles (Wang *et al*., 2004) and quartiles (Seow *et al*., 1998; Fowke *et al*., 2003). However, there was no mentioning about the basis of choosing which cut off point either using tertiles, quartiles or median. The most logical explanation was to be based on normality of the distribution or established and meaningful cut off point from nutritional point of view which was currently unavailable. Perhaps it is time for researchers to come up with a reliable and meaningful cut off point for the dietary ITC intake which could be applicable in all studies.

The median value of dietary ITC intake per 1000kcal was found to be only slightly higher among the cases than the controls, thus was not statistically significant ($p = 0.671$). However, this was not supported by Seow *et al*., (2002) which showed in her study that the dietary ITC intake was higher among the controls than the cases. When dichotomized into high and low intake, the proportion of dietary ITC intake level among the cases and the controls was also not significantly different from one another ($p = 0.645$). The proportion taking high dietary ITC intake was found to be slightly lower among controls than cases. On the contrary, a study done by Zhao *et al*., (2001) among the Chinese women in Singapore, showed that more controls with high consumption of ITC than the cases. This could possibly happen due to few factors such as the arbitrary median cut off point that categorized the dietary ITC intake into high

and low intake and also different methods used to assess level of dietary ITC intake in different studies done.

As for the *GSTs* polymorphism, no significant difference was noted in the distribution of *GSTM1* and *GSTT1* genotype among the cases and controls (p = 0.746, 0.831). The proportion of control having *GSTM1* null genotype (56.0%) in this study was found to be the same as in a study from Italy by Capoluongo *et al*., (2006). However, when compared to other Asian countries, there were more controls with *GSTM1* null genotype which may reflect the population observed in this study. However, assessing across countries revealed that occurrence of *GSTM1* null genotypes are relatively higher in Caucasian compared with Asian population.

The distribution of the *GSTT1* null genotype (41.4%) among the controls was not reflective to any of the *GSTT1* null distribution of different ethnicity based on the multiracial population study from Singapore (Lee *et al*., 1995). In his study among the normal population, the *GSTT1* null distribution among the Chinese, Malays and the Indians were 58.0%, 38.0% and 16.0% respectively. Perhaps for this present study, the *GSTT1* null genotype distribution obtained represented all three ethnicity in this population. For the distribution of *GSTP1* genotypes, no significant difference was detected among the cases and the controls (p = 0.100). This result was further confirmed by studies in France, Japanese and Korea (Jourenkova-Mironova *et al*., 1999; Morita *et al*., 1999; Cho *et al*., 2006) which also demonstrated no significant difference in the *GSTP1* genotypes among the cases and controls.

When the *GSTM1* and *GSTT1* genotypes combined, the difference was still not statistically significant (p = 0.851). This finding was supported by several studies

(Hung *et al*., 1997; Oude Ophuis *et al*., 1997; Kietthubthew *et al*., 2001), with only one study by Gronau *et al*., (2003) stating otherwise.

The distribution of the combined *GSTM1, GSTT1* and *GSTP1* genotypes in this study revealed that there is no significant difference between the cases and controls ($p = 0.242$). Interestingly, it was observed that the distribution of the combined genotypes among the cases and controls showed a less non-significance as compared to the individual genotypes. This suggests a pattern towards significant association if perhaps bigger samples size were employed in the study. To date, only limited numbers of studies have looked into the combination of these three genotypes even in the normal population.

5.3 Socio-demographic profiles and oral cancer

This study used logistic regression which yielded OR to measure risk assessment. Under the role that OR closely approximate to the relative risk when the occurrence of disease in a study population is less than 10%, the interpretation of OR in this study would be similar to the relative risk which was risk of having the disease among exposed than that among not exposed. Oral cancer fall into this category since the prevalence of this disease is 0.04% (Zain *et al*., 1997).

In this present study, oral cancer tended to occur among the older subjects at the average age of 59.6 years. It was pointed out that the older subjects had 1.13 times the risk of having oral cancer than the younger subjects. This was in accordance with the second report of the NCR 2003 which also reported that for both gender, the higher

incidence rate falls in the aged group 50+ years with age specific cancer incidence of male and female were 20.2 and 29.8 per 100,000 population, respectively (Lim and Halimah, 2004). This finding also holds true for many other types of cancers. Best possible explanation is older people probably had been longer or cumulative exposed to risk factors compared to younger people therefore have higher risk to develop cancer.

On the association between gender and oral cancer, females was found to have 74% increased in risk of having oral cancer with an OR of 1.74 (95% CI 1.027 – 2.941). Perhaps this was reflected in the female composition of this study which consists of the higher number of females among the cases and associated with betel-quid habit. This result was also in line with the second report of the NCR 2003 where female also had the highest incidence of mouth cancer with an ASR (age standardized incidence rate) of 2.1 per 100,000 of the population compared to male with 1.6 per 100,000 population. The male to female ratio of oral cancer incidence is 1:1.3 (Lim and Halimah, 2004). Future studies should consider looking into the possible explanation on what factors make females and males differ in their risk of having oral cancer since it can help to narrow down our prevention campaign focusing only to the associated factors.

For this present study, some interesting results were discovered when comparing the association of ethnicity with oral cancer. Chinese was used as the reference group because study by Zain *et al*., (1997) found that the Chinese have a lower risk of oral cancer than Indian and also show a later age of onset. Referring to the Chinese, the Malays had 0.26 times the risk of having oral cancer (OR 0.26, 95%CI 0.083 – 0.838). It also seemed that the Indigenous groups somehow demonstrated lower risk of having oral cancer with an OR of 0.13 (95% CI 0.044 – 0.397). Because these

results were contrary with the findings that Indigenous people of Sabah and Sarawak who were identified as a high risk of developing oral cancer (Zain *et al*., 1997), this results were analyzed again using multivariate logistic regression analysis to control the possible confounding factors which may gives rise to this significant result. Despite the Indian ethnic was also identified as a high risk group (Ramanathan and Lakshimi, 1976), however, Indians were not associated with oral cancer. Perhaps these inconsistencies were due to the different lifestyle practiced and diet consumed by different ethnicity. However, this finding should be interpreted with caution since the possibility of interaction between ethnicity and other factors such as age, drinking and betel quid habits was not properly investigated as they were not the factors under interest of this study. The reduced risk of being Indigenous and being Indian and not associated with oral cancer could be due to the effect of other extraneous factors. On the other hand, the established effect of the ethnicity has on oral cancer also could be due to the common practiced risk habits among certain ethnicity such as betel quid chewer among Indians and Indigenous and not purely attributed to ethnicity. In other words, perhaps the ethnicity is not the factor associated with oral cancer risk but rather the practicing risk habits accompanied these ethnic.

In this current study, alcohol consumption was found to be strongly associated with oral cancer. These alcohol drinkers could possibly confer a 6.93 times the risk of having oral cancer than non-drinkers (OR 6.93, 95% CI 3.065 – 15.656). Since the drinking alcohol is prohibited among the Muslims, the drinking habits probably are more prevalent among the Indians, Chinese and the Indigenous people of Sabah and Sarawak. In fact, the highest prevalence of alcohol drinking habit was found to be among the Indians (13%), followed by the Indigenous people of Sabah and Sarawak (10%) and the Chinese (7.8%) (Zain et al., 1997). According to Zain *et al*., (1997),

there is also a trend showing that alcoholic drinking habits are higher in men than in women especially among the Indians and the Chinese. Another point to note is because tobacco smoking may be a determinant of oral cancer, and because drinkers of alcoholic beverages tend to be smokers, smoking and drinking will need to be evaluated concurrently. The multiplicative interaction indicates that much of the effect of alcohol is via an enhancement of tobacco's effect on these tumors and that reduction in either one of the exposures will substantially reduce cancer incidence (Blot, 1992). Perhaps these also could explain the reason alcohol was strongly associated with oral cancer in this study since in total Indian, Chinese and Indigenous formed more than 75% of the cases and especially when Indian was twice in cases than controls.

Betel-quid chewing habit was found to have the strongest association with oral cancer. The betel-quid chewers tend to have 16.94 times the risk of having oral cancer than those who do not chew (OR 16.94, 95% CI 7.560 – 37.965). Perhaps the majority of the betel-quid chewers were females of the Indians or the Indigenous people of Sabah and Sarawak. This was proven as the high number of betel-quid chewers among the cases seemed to commensurate with the high number of females among the Indians and the Indigenous as compared to the other ethnic groups. This finding is similar to a previous study by Ramanathan and Lakhsmi (1976) which reported betel-quid chewing as being the most important single habit associated with cancerous lesions. In fact, this betel-quid chewing habit still widely practiced and indulged by Indians and the Indigenous people of Sabah and Sarawak (Zain *et al*., 1997). In a Taiwan study, found that betel-quid chewing habit may also increase the risk of developing oral cancer by 62.5 times than among those non-chewers (Hung *et al*., 1997).

Tobacco smoking is a well known major risk factor for oral cancer (Zain *et al*., 1999). The present study showed an OR of 0.88 (95% CI 0.503 – 1.498) for smoking habit, which was not associated with oral cancer and was similar to the odds ratio reported in the Indian populations (Nair *et al*., 1999). In fact, in this study, population mainly composed of non-smokers which are comparable with the study by Zhao *et al*., (2000). These non-smokers reflect the higher feminine gender composition and thus it makes sense to reveal that smoking habit often associate with the masculine gender. Being an established risk factor for oral cancer, the risk reduction indicated by the OR of smoking habit in this study even though non significant was possibly due to the classification of smoking into never smoker and smoker. As duration and amount of cigarettes smoke may play strong role in influencing oral cancer risk, study should look into the details of these. However, it was not achieved as the information in OCRCC database has not been fully completed. It is strongly recommended that future studies not to overlook these details since it may pose a risk. It is also wise to stratify the analysis looking into smoking and its association with oral cancer risk by other risk habits such as drinking alcohol and betel quid chewing. This is especially when knowing the fact that smoking could pose risk when acting synergistically with alcohol or betel-quid.

Subjects with family history of cancer were also found as not associated with oral cancer (OR 1.37, 95% CI 0.668 – 2.814). Perhaps a bigger sample size could be employed in future study to validate whether genetic may play a role in developing oral cancer among those with family history of cancer.

5.4 Dietary ITC intake and oral cancer

Besides tobacco smoking, alcohol consumption and betel-quid chewing, diet and nutrition such as high intake of vegetables and fruits are among the factors most strongly associated with oral cancer. The present study utilizes cruciferous vegetables to represent the primary source of ITC exposure in humans, and Chinese people are among the most frequent consumers of cruciferous vegetables in the world (Seow *et al*., 1998). Among this study subjects, the median dietary ITC intake was 2.31µmol/1000kcal. In the Singapore study (Seow *et al*., 2002), the median dietary ITC intake was 5.16µmol/1000kcal, slightly double the level in this study. The difference again could be due to different methods of ITC assessment, or perhaps a true difference because Singapore consists of more Chinese who eat more vegetables compared to multiracial Malaysian. However, the dietary ITC intake per 1000kcal was not associated with oral cancer risk (OR 1.00, 95% CI 0.965 – 1.034). When the dietary ITC intake was categorized into high and low levels, high level of dietary ITC intake seemed to confer about 13% increased in oral cancer risk (OR 1.13, 95% CI 0.674 – 1.891). However, this finding was not statistically significant. It could be due to many confounding factors such as ethnicity which leads to various diets and lifestyles and as well as different practicing habits. Another possible explanation could be due to a small sample size used in this study which will only able to detect the big difference in the oral cancer risk due to dietary ITC exposure among the cases and the controls (OR 0.45). Perhaps bigger sample size could be employed in future study so that a small difference in the OR would be detectable. This may ultimately lead to a significant difference between high dietary ITC intake and oral cancer risk. Besides, another reason for the statistically insignificant result was the use of semi quantitative FFQ in this study. In this FFQ, the bioavailability of the consumed dietary ITC in our body was

not an accurate measured. In fact, the purpose of the FFQ is to obtain the frequency of the food intake for the subjects based on the past one year in which it may cause biasness during recalling.

While this study reveals that there is no association between the high dietary ITC intake and oral cancer risk, other studies on different cancers suggest otherwise. Several investigations suggest that cruciferous vegetable consumption reduces the risk of lung, colon, bladder, prostate and breast cancer (Verhoeven *et al*., 1996; Michaud *et al*., 1999). In one study by Verhoeven *et al*., (1996) suggests that consumption of cruciferous vegetables, in particular, reduces lung cancer risk. Even though other substances are likely to contribute to lung cancer prevention, cruciferous vegetables are distinguished by their ITC content (Verhoeven *et al*., 1996). Thus, his finding based on a biological marker of ITC provides direct evidence that ITCs in themselves could be important in reducing the risk of lung cancer in human beings.

Zhao *et al*., (2001) also demonstrated a significant association between dietary ITC intake and lung cancer risk. In his study, it was found that ITC has a stronger effect among smokers. It is not surprising, as consistent evidence showed that ITCs are known to reduce lung carcinogenesis by tobacco-related carcinogens. Polycyclic aromatic hydrocarbons such as benzo(α)pyrene and NNK, a tobacco-specific nitrosamine, require metabolic activation. Agents such as ITC, which decrease formation of the electrophilic DNA binding intermediates, reduce DNA damage and thereby inhibit carcinogenesis. Mechanistic studies have shown that this chemopreventive activity is attributable to the inhibition of phase I enzymes and the induction of phase II enzymes (Zhang and Talalay, 1994; Hecht, 1999).

The inconsistencies of the findings could also be attributed again to unstandardized methods used to assess dietary ITC level and also the arbitrary median or quartiles cut-off point used that differed in various studies (Seow *et al*., 1998; Spitz *et al*., 2000; Zhao *et al*., 2001; Seow *et al*., 2002; Fowke *et al*., 2003). If an established cut-off point is used to indicate high and low ITC intake, perhaps the comparison and determination of dietary ITC intake would be more meaningful.

5.5 GSTs polymorphisms and oral cancer

The association between specific *GSTs* polymorphism and risk of oral cancer is a widely explored area of research. Since it is unlikely that any single genetic marker would completely explain the cancer risk in an individual, studying a wide array of susceptibility genes is expected to yield a more complete picture of an individual's cancer risk profile. *GST* catalyze the conjugation of glutathione to several electrophilic compounds, including carcinogenic polycyclic aromatic hydrocarbons and cytotoxic drugs. Conjugation of these agents renders them harmless and enhances their excretion (Zhong *et al*., 2006).

In this study, there were more *GSTM1* null as compared to *GSTM1* non-null among cases. Subjects with *GSTM1* null genotype seems to have 8% decreased in risk of having oral cancer with an OR of 0.92 (95% CI 0.546 – 1.542). However, this finding was not statistically significant. Although there is no definitive explanation for this observation, it was found that smoking and *GSTM1* gene deficiency may interact biologically in a way which enhances the risk for both cases and controls (Kihara *et al*., 1997). The relative importance of smoking might have been reduced in controls

because of the potential involvement of factors other than smoking such as viral or dietary factors, while the relative importance of overall *GSTM1* gene deficiency might have been diminished in cases because of the age-dependent distribution of *GSTM1* null genotype which was low in the older age group. It may also be that potential compensation through the activities of other GST enzymes is enough to totally overcome the effect of *GSTM1* deficiency.

The observed lack of an association between the *GSTM1* genotype and susceptibility to oral cancer in this study is similar to the observed in previous studies (Park *et al*., 1997; Hung *et al*., 1997; Oude-Ophuis *et al*., 1998; Jourenkova-Mironova *et al*., 1999; Morita *et al*., 1999; Olshan *et al*., 2000; Sreelekha *et al*., 2001; Hahn *et al*., 2002; Gronau *et al*., 2003; Sikdar *et al*., 2004; Sugimura *et al*., 2006; Sharma *et al*., 2006). On the contrary, studies by Buch *et al*., (2002), Sato *et al*., (1999), Kiettubthew *et al*., (2001), Cha *et al*., (2007) and Kihara *et al*., (1997) had showed that deficiency of GSTM1 is associated with increased risk for major smoking-related cancer such as oral cancer in the Asian populations. Meanwhile among the Caucasian populations, only studies by Trizna *et al*., (1995) and Capoluongo *et al*., (2006) found that there is association between the *GSTM1* null genotype with oral cancer risk.

As for the *GSTT1* null genotype, it has been implicated in increased susceptibility to both lung (Chan-Yeung *et al*., 2004) and cervix (Sobti *et al*., 2006) cancer. However, the association between the *GSTT1* null genotype and increased risk for oral cancer as reported in studies by Jourenkova-Mironova *et al*., (1999) and Sharma *et al*., (2006) were not confirmed in the present study. Subjects with *GSTT1* null genotype posed to be 0.94 times lower risk of having oral cancer than those possess *GSTT1* non-null genotype (OR 0.94, 95% CI 0.559 – 1.597), although there

was no significant association. The discrepancy between the studies may be due to several factors, including possible differences in the study populations (for example, Caucasians and Indian population in Jourenkova-Mironova and Sharma studies respectively as compared to the multi races of Malays, Chinese, Indians and Indigenous in the current study) and, perhaps most importantly, the studies may be using different criteria for case definition such as oral and pharyngeal cancer by Jourenkova-Mironova *et al*., (1999) and oral squamous cell carcinoma in the present study.

In the present study, it was demonstrated that there is no association between the polymorphism *GSTP1* genotype and risk for oral cancer. As compared to the *GSTP1* wild-type genotype, subjects with *GSTP1* polymorphism was observed to have 35% less oral cancer risk (OR 0.65, 95% CI 0.385 – 1.088). A potential confounder for the analysis of *GSTP1* genotype in oral cancer may be due to smoking status of the subjects. Because *GSTP1* gene has the highest affinity to detoxify the carcinogens from the cigarette smoke such as PAHs, subjects with low activity or *GSTP1* polymorphic genotype will not have the capacity to eliminate the carcinogens effectively. Perhaps detailed information on smoking status such as the duration and the number of stick smoke per day would be helpful to observe the high or low exposure of the *GSTP1* gene with oral cancer risk. In fact, a study by Park *et al*., (1999) indicated that *GSTP1* polymorphism was found to confer higher risk for oral cancer among the light smoker. Since our smoking data were just categorical and the number of smokers in this study was only about 30%, thus it may suggest the insignificant association between *GSTP1* genotype and oral cancer risk. There is also possibility that *GSTP1* genotype effect on oral cancer risk was modified by dietary ITC if there is interaction occurs between the gene and ITC which should be further investigated in future studies.

Many studies were also conducted to look into the association of *GSTP1* and cancer risk. Among the Asians, there were a few studies linking *GSTP1* genotype with risk for cervix (Sobti *et al*., 2006), breast (Egan *et al*., 2004) and bladder (Srivastava *et al*., 2005) cancer. Only one study by Miller *et al*., (2003) found association between *GSTP1* genotype with risk of lung cancer among the Caucasian. Together, these studies implicate *GSTP1* as a major carcinogen-detoxifying enzyme in various human tissues and suggest that diminished *GSTP1* activity may play an important role in risk for oral as well as other cancers.

On the contrary, fewer studies have reported on the association between the *GSTP1* polymorphism and oral cancer susceptibility. Some links had been suggested between this polymorphism and increased oral cancer risk in Japanese (Katoh *et al*., 1999) and Indian population (Sikdar *et al*., 2004). Based on a meta-analysis, none of the Caucasians population had shown any linkage between the *GSTP1* polymorphism and susceptibility to oral cancer (Hashibe *et al*., 2003). Only one study by Jourenkova-Mironova *et al*., (1999) in France showed some significant association between the *GSTP1* polymorphism and oral cancer risk.

In this study, the polymorphism of the combined *GSTM1* and *GSTT1* gene were not associated with oral cancer. In other words, this *GSTs* polymorphism may not be a risk factor in oral cancer. In fact, subjects with the combined *GSTM1 and GSTT1* polymorphism genotype had a reduction in risk by 5%, although this is not statistically significant. However, from a previous meta-analysis study (Ye *et al*., 2006), it was found that the joint effect of both *GSTM1* and *GSTT1* null genotypes was associated with the risk of head and neck cancer with an OR of 1.99 (95% CI 1.74 – 2.24). On the contrary, Hung *et al*., (1997) in his study revealed that polymorphism of the combined

GSTM1 and *GSTT1* was not associated with oral cancer despite having the OR of 3.1 (95% CI 0.9 – 11.0). This result was also supported by Kietthubthew *et al*., (2001) where no association was found between the combined *GSTM1* and *GSTT1* null genotypes with oral cancer (OR 2.0, 95% CI 0.5 – 7.8).

The effect of no association between combined *GSTM1* and *GSTT1* and oral cancer risk may be masked by interactions with confounding factors such as other polymorphic loci encoding detoxifying enzymes. This study data show no influence of the combined *GSTs* on risk of oral cancer. Various factors including exposure and diet will influence the importance of allelism. Dietary factors are also likely to be critical because of their effect on DNA damage, mutation and repair (Deakin *et al*., 1996). Perhaps testing several genetic polymorphisms simultaneously has the potential to identify individuals with extremely high cancer risk. This has profound implications for prevention, since such high-risk individuals may be intensively screened as well as potentially treated with novel chemo-preventive approaches (Trizna *et al*., 1995).

In this present study, it was also confirmed that there is a lack of association between *GSTM1, GSTT1* and *GSTP1* polymorphism genotype and oral cancer risk. Interestingly subjects with combined *GSTM1, GSTT1* and *GSTP1* polymorphism genotype had a 36% reduction in risk of having oral cancer. It was observed that neither *GSTM1* or *GSTT1* nor *GSTP1* was associated with oral cancer when analyzed separately, however it was noted there is a pattern towards statistical significance association between the combined *GSTM1, GSTT1* and *GSTP1* polymorphism genotype and oral cancer. This pattern is similar to that observed in previous study examining polymorphic genotypes and esophageal cancer risk (Jain *et al*., 2006). Another study found that combined *GSTM1, GSTT1* and *GSTP1* polymorphism was associated with

an increase risk of developing cervix cancer among the passive smokers (Sobti *et al*., 2006). So far, there is no study done on the combined *GSTM1, GSTT1* and *GSTP1* genotype with oral cancer risk.

It has been suggested that genetic variations in the ability to metabolize tobacco smoke carcinogens are most important in determining cancer risk at low levels of exposure, and may be less relevant at higher smoking doses where high levels of carcinogen exposure overwhelm polymorphism-induced differences in enzyme activity and/or expression (London *et al*., 1995). Therefore, it seems more appropriate to examine the combined effects of polymorphism genotypes of *GSTM1, GSTT1* and *GSTP1* rather than the independent effect of each polymorphism genotype, because they are involved in the detoxification of the tobacco-related carcinogens (Hung *et al*., 1997).

Besides, studies have indicated that the risk of cancer increases with the number of *GST* variant alleles (Cote *et al*., 2005). We found that carriers of two to three polymorphism *GSTs* genotypes seemed to be at higher risk of cancer than carriers of no or one polymorphism *GSTs* genotypes. A possible explanation is that the presence of only a few variant *GST* alleles will not result in a decreased detoxification of carcinogenic compounds because other *GSTs* with similar substrate specificities can compensate. However, if two or more *GSTs* are either lacking or have decreased activity then the detoxification will be insufficient and the risk of cancer will increase (Sorensen *et al*., 2006).

Several recent studies have linked GST genotypes to cancer risk. Specifically, *GSTM1* and *GSTP1* are known to be active against epoxides of carcinogenic

polyaromatic hydrocarbons. The cancer protective effects of *GSTT1* have been attributed mainly to its role in the detoxification of environmental xenobiotics. From this current study, it suggests that there is no significant difference for *GSTM1, GSTT1* and *GSTP1* polymorphisms and risk of oral cancer. These could be due to the small sample size employed in this study. When sample size estimation was conducted for this study, estimation was done for all objectives that have information needed available and the dietary ITC has yielded the largest affordable sample size. Unfortunately, at this stage, the information needed to estimate adequate sample size for *GST*s prevalence was not yet retrievable. Only at later stage when the study was already completed that we managed to obtain information from valid resources. Therefore, perhaps a bigger sample size should be employed in future studies to be able to detect a small difference in the OR of the exposure among the cases against the exposure among the controls. Despite the inconsistency of this result comparing with other studies, it was observed that there is a pattern towards significant association between the *GSTs* polymorphisms and the oral cancer risk. Another possible explanation could be the lack of diet high in antioxidant and ITC among the *GSTs* polymorphisms subjects. Consumption of sufficient diet rich in antioxidant certainly will be able to eliminate the harmful free radicals or reactive oxidative species (ROS) from our body. Insufficiency intake of dietary ITC among the *GSTs* polymorphisms subjects may experience less *GST* induced from ITC which eventually lead to inefficiency of elimination of high level of carcinogens from the body.

5.6 Dietary ITC intake, GSTs polymorphisms and oral cancer

In this study, Cochrane-Mantel Haenszel analysis was done to look into association between dietary ITC and oral cancer risk when *GSTs* polymorphisms were controlled. The common OR yielded indicated the risk of having oral cancer in individuals with high dietary ITC in relative to the low dietary ITC intake when the particular *GST* was controlled. If the OR for *GST* non-null/wild-type was similar to null/polymorphism genotypes but differ from common OR then it suggested that the association between the dietary ITC and oral cancer was confounded by the particular *GST*. In case where one of the stratified OR differ from common OR, and also differ from one another, it indicated that an interaction may has occurred between the particular GST and dietary ITC, thus influence the association between dietary ITC and oral cancer risk.

This case-control study evaluated the role of cruciferous consumption in individuals with the *GSTs* polymorphisms genotypes, and compared the incidence of oral cancer in various dietary groups. Unfortunately both the dietary ITC and *GSTs* polymorphisms had no significant association with oral cancer risk. When stratified using Mantel-Haenszel analysis, the OR for the *GSTM1* null genotype in the presence of high ITC intake was 1.10 (95% CI 0.545 – 2.196). Although consumption of high ITC among the *GSTM1* null genotype seems to confer 10% increase the risk of having oral cancer, it was not statistically significant. In this study, it was also observed there is no statistically significant interaction between dietary ITC intake and the *GSTT1* genotype. Among individuals with the *GSTT1* non-null genotype, higher dietary intake of ITC was associated with a 23% increased in oral cancer risk (OR 1.23, 95% CI 0.627 – 2.399), whereas among individuals with the *GSTT1* null genotype higher ITC intake

was associated with no change in risk (OR 1.00, 95% CI 0.443 – 2.239) but again these were not significant.

Indication of no interaction between dietary ITC intake and *GSTM1* and *GSTT1* polymorphism with the oral cancer was noted as the OR for *GSTM1* and *GSTT1* null and their non-null are similar with the common OR. However, it should be further investigated with larger sample size since all were found not significant in the present study. It also suggests that there was possibility of no evidence that effect of high dietary ITC intake on oral cancer was modified by *GSTM1* or *GSTT1* deletion which needs to be confirmed in future studies.

No information on the specific role of the *GSTP1* gene in ITC metabolism has been reported. Interestingly, this study suggests that *GSTP1* may be a key enzyme in the metabolism of ITCs in human. When stratified by *GSTP1* genotype, a protective effect of high ITC intake was observed only among subjects with the *GSTP1* polymorphism genotype (OR 0.80, 95% CI 0.387 – 1.635). Although this finding was not significant, there could be a possibility of the interaction between the dietary ITC intake and *GSTP1* polymorphism with oral cancer due to the OR for each of the *GSTP1* genotypes differ from one another and also differ from the common OR.

When the association between dietary ITC intake and oral cancer was stratified by combined *GSTs*, it seems there is protective indication among the wild-type genotype as compared to the polymorphism genotype when stratified analysis were done on *GSTs* separately. The OR for the combined *GSTM1* and *GSTT1* polymorphism genotype in association with high ITC intake was 1.21 (95% CI 0.659 – 2.215) and it was not statistically significant. Again because the OR for the stratified genotypes of

combined *GSTM1* and *GSTT1* was similar to one another and close to the common OR, there were no interaction between the combined *GSTM1* and *GSTT1* polymorphism and dietary ITC intake in relation of oral cancer.

Among the combined *GSTM1, GSTT1* and *GSTP1* genotypes, high intake of dietary ITC conferred about 4% reduction in risk of having oral cancer in subjects with combined *GSTM1, GSTT1* and *GSTP1* wild-type genotype (OR 0.96, 95% CI 0.210 – 4.421). However, it was not statistically significant. This finding suggests that there was no significant association observed between the dietary ITC and oral cancer risk among combined *GSTM1, GSTT1* and *GSTP1* polymorphism individuals.

In summary, among the study subjects, there was no significant association observed between dietary ITC intake, *GSTs* polymorphism and oral cancer risk. However, an interaction was found between *GST* genotype and dietary ITC such that high dietary ITC is associated with a possible lower risk of oral cancer among individuals who are having polymorphism of *GSTP1* and hence metabolize and excrete these compounds at a slower rate. Nevertheless, this association was not statistically significant. These observations could be due to the median values for consumption of cruciferous vegetables in this study population may not be sufficiently high to substantially elevate ITCs to the necessary levels in order to observe the benefit of reduced ITC excretion with the *GSTM1* and/or *GSTT1* null genotype, especially in the presence of elevated exposure to tobacco carcinogens.

There are some issues that should be considered in evaluating *GST* genotypes as an independent risk factor for oral cancer, and these may also explain the lack of consistency between studies. At the target tissue level, it is probable that

biotransformation ultimately depends on a delicate balance between phase I and II enzymes. In addition, among various isoenzymes of the *GST* family, each may compensate to some degree for reduced activity of another, such that the effect of individual genotypes is indiscernible (Seow *et al*., 2002). An absence of the high activity allele leads to a reduction in, rather than absence of activity, which may again be difficult to demonstrate in epidemiological studies.

Besides, there has been some uncertainty as to whether the protective effects of cruciferous vegetables can be attributed to individual compounds like ITC or indoles, or if they are due to the action of other unknown chemicals (Steinkellner *et al*., 2001). The present study suggests that ITCs are indeed the major constituents in cruciferous vegetables that account for their chemopreventive activity in the oral cancer. It shows that the ITC-oral cancer effect is strongest among individuals with low activity (polymorphism) for *GSTP1*, one of the major metabolic pathways for elimination of ITCs. Similar relationships between GST, ITC and lung cancer have been demonstrated in diverse population (London *et al*., 2000; Spitz *et al*., 2000; Zhao *et al*., 2001). Taken together, these results provide strong evidence that the inverse ITC-cancer relationship is a causal one.

Cruciferous vegetables contain high concentrations of the anticarcinogenic compounds isothicyanates (ITCs) (Hecht, 1999), that are believed to reduce risk of cancer through an induction of phase II enzymes and, in turn, phase II enzymes conjugate ITCs leading to a faster detoxification of carcinogens (Zhang and Talalay, 1998). For ITC to play an active role, it very much depend on *GSTs*. If the *GSTs* are not present/in low activity, than ITC may play a more vital role in protecting the cells from the carcinogens. If this is true then intake of ITCs should reduce the risk only among

individuals with *GSTM1* and/or *GSTT1* genotype. The discrepant results could be explained by different study populations, differences in assessment of intake of cruciferous vegetables, other compounds than cruciferous vegetables could interact with the *GSTs*, and lastly, this finding could be a chance finding because the power for investigating interactions was too small.

For the *GSTP1* polymorphism, it was found that high dietary intake of ITCs seem to reduce the risk of oral cancer. Part of the observed effect could be explained by the high content of antioxidants found in fruit and vegetables. *GSTP1* is the most abundant *GST* in the oral (Sarkar *et al*., 1997) and a decrease in enzyme activity among carriers of the variant *GSTP1* allele could theoretically results in an oxidative stress. A high intake of dietary antioxidants could possibly prevent this. No other study known to us has investigated the interaction between *GSTP1* and intake of ITCs in relation to oral cancer. Interactions between intake of ITCs, the *GSTP1* Ile105Val polymorphism and colorectal cancer have been indicated by some studies but the results are inconsistent (Seow *et al*., 2002). All in all this study results suggest that there are some interactions between the *GSTs* polymorphisms and intake of ITCs, though larger studies are needed before conclusions can be made.

ITCs are potent inducers of *GSTs* (Hecht, 1999), which complicates mechanisms of biological interaction between ITCs and *GSTs* (Meyer *et al*., 1995). Further, given the multiple anticarcinogenic actions of ITCs, including inhibition of carcinogen activation by cytochrome P450 enzymes (Hecht, 1999), induction of apoptosis and protection against oxidative damage, it is likely that additional mechanisms contribute to the protective effect of ITCs seen primarily among individuals deficient in *GSTM1* and *GSTT1*. There is much that is not known about ITC

metabolism and its biological effects. Nonetheless, evidence for the chemopreventive potential of ITCs is compelling (Hecht, 1999).

Modification of ITC chemoprevention of oral cancer by polymorphisms of *GSTM1, GSTT1* and *GSTP1* is biologically plausible. *GST*-catalyzed conjugation with glutathione aids in elimination not only of environmental carcinogens but also of anticarcinogenic substances in the diet, such as ITCs. Conjugation of ITCs with glutathione, a reaction catalyzed by *GSTs*, constitutes the main route of ITC metabolism (Conaway *et al*., 2002). Among four *GSTs* studied in vitro (*GSTM1, GSTP1, GSTA1* and *GSTM4*) for their catalytic properties with respect to GSH conjugation of 14 different ITCs, *GSTM1* was the most efficient (Kolm *et al*., 1995). Further, ITCs are among the *GST* substrates that are most rapidly conjugated (Kolm *et al*., 1995). While *GSTT1* has not been studied in vitro, we found that *GSTT1* is important in ITC conjugation in humans (Seow *et al*., 1998).

There are some studies which showed the association of dietary ITC, *GSTs* polymorphism and risk of oral cancer. In a study done among Singapore Chinese, Seow *et al*., (2002) demonstrated a significant inverse association between cruciferous vegetable intake and colorectal cancer (OR 0.43, 95% CI 0.20 – 0.96). While there is now a body of evidence that supports the association between cruciferous vegetables and colon cancer (Seow *et al*., 2002), the present study provides new information on the effect of GST, the main metabolic enzymes, on this relationship, and is the first to demonstrate this using ITC values calculated from the full range of cruciferous vegetables consumed in the population.

In the study by Wang *et al*., (2004) found that greater reduction in lung cancer risk among non-smokers for high cruciferous intake among *GST* null or double null genotypes rather than among *GST* present genotypes. This suggest that the *GST* null genotype does allow a benefit from a slower rate of ITC excretion, even at the lower absolute cruciferous intake levels found in Western diets, but only among individuals without any or very little tobacco exposure.

Zhao *et al*., (2001) in her study described an inverse association of dietary ITCs on lung cancer risk among Singapore Chinese women, which is modified by *GSTM1* and *T1* genotypes. Those with the null genotype for either or both enzymes experienced a significant reduction risk with higher intake of ITCs, but the effect was smaller and not statistically significant if either or both genes were present. Also was reported for the first time, a modifying effect of the *GSTM1* genotype on the effect of ITCs in lifetime nonsmokers.

London *et al*., (2000) showed that, among Chinese men in Shanghai, individuals with detectable urinary ITCs had a significantly reduced risk of lung cancer, and that this effect was primarily confined to inidividuals with *GSTM1* or *T1* (or both) null genotypes. In fact, Spitz *et al*., (2000) found that a combination of low ITC intake and *GSTM1* and *T1* null genotypes conferred the highest risk of lung cancer among smokers.

The strengths of this study are that dietary information was collected using a validated questionnaire which included some major cruciferae consumed in this population, allowed computation of ITC intake and adjustment for total energy and other relevant variables. It was also noted that the distribution of ITC intake among

controls is not dependent on *GST* genotype and does not explain the effect modification observed. It was also observed a stronger inverse association with oral cancer risk for high dietary ITC intake among *GSTP1* polymorphism individuals, but not among *GSTP1* wild-type individuals. Previous study reported a greater inverse association among *GSTP1* polymorphism individuals, supporting the hypothesis that *GSTP1* polymorphism individuals receive the greatest protective effect of high ITC intake (Seow *et al*., 2002).

5.7 Limitation of the study

The chief limitation in this study is the relatively small number of sample size which could not reflect the true population but rather confined to this study. This is because the sample size was calculated based on the objective dietary ITC and oral cancer risk and as well as the feasibility to conduct the study within the limited resources and time constraint. Perhaps this set of novel findings can only be verified when a corresponding larger number of sample size has been achieved.

None of the studies conducted to date have been able to assess gene-environment interaction with precision due to limited statistical power. In addition to adequate sample size, assessment of gene-environment interaction also depends upon the accurate and detailed measurement of exposures and the proper statistical evaluation of interaction on the multiplicative and additive scales (Geisler and Olshan, 2001). In general, most case-control studies will require a total sample size of approximately 1,000 persons to achieve 80 percent power (Geisler and Olshan, 2001). This big number was not feasible due to time and financial constraint.

Case-control studies with small sample size may be reporting inflated ORs. These results suggest caution in the interpretation of small case-control studies. The summary ORs for *GSTM1*, *GSTT1* and *GSTP1* may also differ by geographic region. The prevalence of these genotypes in controls varied widely among and within regions. It will be of interest to further explore whether these genotypes are more relevant in specific ethnic groups, with respect to the risk of oral cancer (Hashibe *et al*., 2003).

Another limitation which requires understanding is that carcinogen metabolism is a complex process, involving the interaction of numerous carcinogens and enzymes. The metabolic action of GST enzymes may differ by cancer site; the highest concentrations of *GSTP1* have been observed in oral and pharyngeal tissues, and highest concentrations of *GSTM1* have been observed in laryngeal tissue, relative to the other *GSTs* (Geisler and Olshan, 2001). GST enzyme expression may also differ according to the general controls of gene expression, such as the rates of transcription, translation and degradation as well as possible posttranslational modifications (Hashibe *et al*., 2003).

Case-control studies are also limited in their ability to accurately collect dietary data from the distant past, when mechanisms for cancer inhibition may be more important. Animal studies suggest that ITCs may inhibit tumor initiation but not tumor progression (Hecht, 1999). It is possible that we have not completely captured cruciferous consumption in the distant past.

There are also inherent limitations in this study, including use of food frequency data to estimate ITC intake and possible recall bias. Dietary ITCs have rarely been

considered as confounding factors in molecular epidemiology studies. Some of the inconsistencies that have been noted in the study of the effect of *GST* genotypes could be due to unexpected confounding factors in the diet. These data highlight the complexity and challenges inherent in the analysis of diet-gene interactions.

Dietary ITC intake differs from one study to another most likely due to the sample size, types and amount of cruciferous vegetables consumed and differences in dietary assessment methods (for example urinary ITC collection). Perhaps the high dietary ITC intake is not high enough to show any significance in this study. Another consideration is the bioavailability of ITC at the target tissue level. The effect of ITC intake may differ among different cancer sites. In addition to ITCs, these cruciferous vegetables also contain many other compounds that are postulated to have protective effects, including carotenoids, vitamin C, folic acid, fiber and protease inhibitors. It is plausible that individuals likely to be at increased risk for lung cancer (current smokers who are homozygous null for protective genotypes) who also consume the least amount of carcinogenic blocking compounds would find themselves in the highest risk category.

Possible explanations for the discrepancy in our findings include unmeasured effects of other genes and polymorphism, differences in absolute levels of cruciferous vegetable or ITC consumption, differences in exposure to tobacco smoke and differences in measured exposure (i.e. cruciferous vegetable vs ITC intake). For example, *GSTM1* combined with other *GST* polymorphism (i.e. *GSTP1*) or alternative gene polymorphisms in the same metabolic pathway (e.g. CYP450; NAD(P)H quinone oxidoreductase, NQO1; N-acetyltransferase, NAT) were not evaluated. Additional

analysis with other relevant genotypes may help clarify the conditions and mechanism for anti-carcinogenic effects of ITCs.

Another limitation was possibility of under reporting the dietary ITC intake. There are a few exotic food items that have to be excluded from being computed into the total energy and daily nutrient intake due to the limitation in NutrieMart software, however, the amount consumed was small and may not give rise to any significant difference in total calorie intake. Nevertheless, we still consider this as limitation and future study is recommended to further look into this matter.