CHAPTER 6

DISCUSSION

6.1 Limitation of study

The aim of this study was to identify risk factors in the development of oral cancer in Jakarta population, Indonesia. It is a hospital-based case-control study where subjects who participated in the study came from five hospitals in Jakarta. Data on risk factors were obtained based on interviews with the subjects using a structured questionnaire as well as an analysis of their blood samples. In undertaking this study, several limitations were encountered such as:

6.1.1 Sample selection

a. Jakarta population

Jakarta is divided into 5 districts. In this study, data were obtained from 5 hospitals representing each of the districts of Jakarta (South, East, North, Central, and West). However, not all of the data were obtained from the 5 hospitals selected during the sample collection period. Fatmawati hospital failed to provide cases because all the cases that were eligible were at the critical stage making interviewing and taking of blood samples difficult. The selection of hospital may cause bias if it is insufficient in representing the distribution by ethnic group in Indonesia. This condition has been handled by choosing the main referral hospital in Indonesia which is located in Jakarta itself as well as the main or the biggest hospital in each district of Jakarta as selected study centers. The distributions of the subject were found to closely reflect the Jakarta population whereby the Deutro Melayu represent the dominant ethnic group followed by Proto Melayu.

b Sample size

Initially, it has been decided that this study was a case-control study matched for age and gender. Based on the formula of matching case-control study, it was calculated that the subject's number should be 89 cases and 89 controls. However, because of the limitation in getting cases which fulfill the inclusion criteria during the study period, it was only possible to obtain 81 cases. Therefore, in order to maintain the power of study to at least 80%, the number of controls was doubled resulting in 81 cases and 162 controls. This resulted in the power of study of 88 %.

c. Control subjects

The selection of comparable control subjects in a case-control study is very important. As stated by Schlesselmen (1982), matching may provide the best means of investigating a very specific hypothesis or postulated mechanism of action, allowing one to conduct a comparatively small study. Matching is undoubtedly the most useful method for small studies, where greatly disparate case-control series may easily arise unless special precautions are taken to assure uniformity on confounding variables.

In this study, age and gender matching was chosen as confounding factors which had to be matched, because it was assumed that age and gender were related to the study exposure, not to the disease. Consequently, the two groups were comparable. As mentioned above, this study utilized a sampling method in which two controls were matched for each case.

The inclusion, exclusion criteria and operational definition criteria of this study were adopted according to case control study criteria by Schlesselmen (1982) and other similar studies done in many countries (Hashibe et al., 2000; De Stefani et al., 2006, Ogden, 2005; Olshan et al., 2000; Nair, 1999; Marchioni et al., 2007). However the use of only hospital-based controls may lead to bias in estimation of OR for life style habit, especially if the researcher had intended to see the association of many suspected risk factors (such as smoking, alcohol drinking, betel quid chewing, and dietary pattern). Furthermore, in terms of genetic polymorphism characteristic of the population, the choice of hospital based control subjects only could also provide bias results in the risk estimation (Odds ratio) if the controls do not reflect the exposure and/or genotype distribution of the population. However, most studies in this area have relied solely on the use of hospital based controls due to the difficulties in sample collection and limited duration of the study. In this study, suitable statistical analyses, such as using case-control matching and conditional logistic regression to minimize the bias of the result have been applied.

6.1.2 Recall bias

Another limitation in this study was that most of the data were collected based on patient recall (such as time start smoking, precise duration of smoking or other habits). Hence, measurement error may result since the reporting of risk habits would depend on the memory of the subjects and their willingness to fully cooperate with the interviewers. Besides that, recall of past dietary intake might be affected by dietary stability, due to loss of memories (dietary habit last 5 years), education level and current food intake. All the above recall biases may influence the validity of results. The longer subjects practice the habits the

bigger the recall bias. In order to minimize bias through differing interview styles, the interviewing was undertaken solely by the author. A simple questionnaire format was also used to facilitate response from the sample, some of whom were illiterate.

6.1.3 Limited reference on diet and nutrition

Another limitation was the very limited information available on diet and nutrition associated diseases especially for cancer or oral cancer in Asian countries and especially in the population of Indonesia to serve as a reference point for this study. Hence this study had relied heavily on Western diet which does not reflect that in Asia and especially Indonesia.

6.2 Hypothesis

Based on the findings of this study:

6.2.1 The following hypothesis are rejected :

6.2.1.1. Smoking is not a risk factor for oral cancer in the Jakarta population.

6.2.1.2. Betel quid chewing is not a risk factor for oral cancer in the Jakarta population.

6.2.1.3. There is no correlation between dietary patterns in development of oral cancer in the Jakarta population.

6.2.2 The following hypothesis are accepted :

6.2.2.1. Alcohol drinking is not a risk factor in development of oral cancer in the Jakarta population.

6.2.2.2. Genetic polymorphism of GSTM1, GSTT1 and CYP1A1 are not risk factors in development of oral cancer in the Jakarta population.

6.2.2.3. There is no correlation between smoking, alcohol consumed and betel quid chewing with polymorphism of GSTM1, GSTT1 and CYP1A1 and the development of oral cancer in the Jakarta population.

6.3 Socio demographic characteristics of Jakarta population

6.3 1. Age and Gender

Globally, it has been well documented that the prevalence of the oral cancer increases with increasing age. The highest prevalence for oral cancer occurs among those above forty years old and it is more prevalent among men than women (Johnson et al, 2003a; Parkin et al., 2005). The same distribution of oral cancer was found in this study population. The mean age of cancer cases was 47.4 years ± 12.4 and men were more affected by oral cancer than women (1.6:1). This finding is similar to that of the World Cancer Report (2003) written by Stewart and Kleihues.

6.3.2 Site of oral cancer

The tongue was the most common cancer site (59.3%) in this study followed by the gum (19.8%), which includes the vestibule and retromolar area with the buccal mucosa being the third most common site. This is consistent with global epidemiology data that showed the lateral border and base of the tongue to be the most “cancer prone” areas and make up the common intra oral sites for

cancer in most populations (Moore et al., 2000b). However the site distribution in this study differed from most of researches done in other Asian countries, whereby the buccal mucosa was the most common site. This could be due to the predominant habit practiced in the study population. The buccal cancer is prevalent in countries where betel quid chewing with or without tobacco is the major habit practiced such as in India, Taiwan, Thailand and Myanmar (Gupta and Ray, 2004; Yang et al., 1996; Reichart, 1995; Reichart and Way, 2006). Betel quid chewing habit in Indonesia has been mainly substituted with smoking habit since early to mid 1900s (Achadi, 2005). This is reflected by the small number of subjects in this study who chewed betel quid.

Furthermore, it also has been suggested that this site predilection (lateral border and base of the tongue) for intraoral cancer is due to the pooling of carcinogens in saliva in these food channels and reservoirs (Chen et al., 1990) or 'gutter zones' (Johnson and Warnakulasuriya, 1993a). The pooling of saliva containing carcinogens, such as benzopyrenes and nitrosamines, in gravity-dependent regions has been proposed to explain this occurrence. Besides the nasopharyngeal area, tongue might also be at risk because they receive a high concentration of carcinogenic-containing tobacco smoke on inhalation. Furthermore, the posterior tongue might be sensitized to the carcinogens by the heat of the inhaled gas stream. The combination of heat and carcinogens has also been proposed to explain the development of lip cancer in pipe smokers. Besides that, the absence of keratin at the ventral of the tongue might further contribute to the vulnerability to these sites to carcinogens (Schmidt et al., 2004).

It has been established that the most commonly cited etiological agents and/or risk factors for tongue cancer is tobacco (smoked or chewed) and alcohol (IARC, 1986). However, additional causative factors including nutritional deficiencies, viruses and the possibility of genetic factors involvement have also been suggested (Macfarlane et al., 1996; Todd et al., 1997). Moore et al. (2000b) stated that in early 2000, among many other Asian countries, the highest prevalence of tongue cancer occurs among males in the Indian subcontinent. However, with the increase in betel quid chewing habits, the high prevalence changed from tongue cancer to buccal mucosa cancer. Such scenario is also found within the Indian sub-population in Singapore (Gupta and Ray, 2004).

6.4 Risk habits in Jakarta population and risk for oral cancer

6.4.1 Smoking habit and risk for oral cancer

Tobacco smoking is the main known cause of cancer-related death worldwide. It has been estimated that tobacco causes approximately 25% of all cancers in men and 4% in women. Furthermore, tobacco smoking is responsible for 91% of all lung cancers in men and 69% in women. Additionally, the proportion of cancers of the esophagus, larynx and oral cavity, which is attributable to tobacco are, between 43 and 60% (Stewart and Kleihues, 2003).

Among the three major habits (smoking, alcohol dinking and betel quid chewing) in case and control groups that were investigated in this study, smoking was the most commonly practiced (54.2% and 46.8% for cases and controls respectively). In this study the tobacco smoking habit was more

prevalent among men (74%) than women (14%), with a ratio of 5:1 and *kretek* was the most preferred type of tobacco consumed (82.1%).

Consumption of tobacco products has increased dramatically as a result of the mass production of cigarettes, not only in Indonesia but all over the world, especially starting in the second half of the 20th century. It is well documented that Indonesia is the second largest market for cigarettes in Asia Pacific and experiencing high growth (Lawrence and Collin, 2004). Based on estimation in 1995, almost 70% of men and 3% of women smoked. The increasing annual rate of smokers in Indonesia is also attributable to the social acceptance of the smoking habit. *Kretek* is the most (88%) common type of cigarettes used in Indonesia (Achadi et al., 2005). Incorporating diverse ingredients, *kreteks* are cigarettes based on a blend of tobacco with cloves and clove oil, lending them a distinctive scent. The anaesthetizing effect of clove oil accounts for their historic use to alleviate sore throats and asthma but also results in high tar yields (2-5 times higher than cigarettes) and potentially damaging to the lungs (Hanusz, 2002). Despite the proclaimed status of *kretek* as a key cultural signifier and powerful symbol of Indonesia, their dominance is a comparatively recent phenomenon and white cigarettes have a longer established presence. Furthermore, the addictive effect of *kretek* is known to be higher than cigarettes. The distinctive scent as well as the high nicotine content (1.7-2.5 mg/stick, normal level is <2.0 mg), makes *kretek* more popular among smokers. Additionally, because of that reason, the smoking of *kreteks* has replaced the habit of betel quid chewing during the early to mid 1900s among many rural males (Achadi et al., 2005). The findings also showed that smoking of pipe or cheroot or the traditional hand rolled cigarettes (*kawung or menyan)* are not

popular. All subjects smoked the established brands of *kretek* cigarettes. However in this study no questions were asked on the usage of tobacco filter.

The relationship of tobacco to cancer has been well documented. Polycyclic aromatic hydrocarbons (PAHs) which are produced during smoking (Roghman et al., 2000) and another class of carcinogens represented in tobacco smoke is N-nitroso compound, particularly the nitroso derivatives of nicotine, aromatic amines, benzene and heavy metals, which is independently established as carcinogenic for humans, are present in tobacco smoke (Stewart and Kleihuess, 2003). All of the chemicals in tobacco smoke play an important role in carcinogenesis. However, there are, very limited studies on the effect of *kretek* cigarettes (which are very popular in Indonesia) to diseases, including oral cancer. However, a study of lung function and bronchodilator test on smokers, ex-smoker and non-smoker done by Bernida et al (1990) found that the mixture of tobacco and cloves may increase the temperature of cigarettes when it is burnt. Furthermore, it increases the level of carbon dioxide and nicotine to three times, as well as the tar content by 5 times. Thus this may lead to higher toxicity to oral mucosa and increase of risk for oral cancer.

This study found that almost all smoking factors (number of sticks per day, duration of smoking, and type of tobacco and pack-years of exposure of tobacco) doubled the risk. Furthermore those who smoked 11-20 sticks of cigarette per day displayed about three-times greater risk for developing oral cancer. Similarly, those who smoked for more than 10 years increased their risk almost three-times. These findings are similar to global features of smoking worldwide (Mehta et al., 1981; Blot et al., 1988; Hashibe et al., 2000; Hindle et

al., 2000a). The only exception for the number of sticks smoked per day, i.e. those who smoked more than 20, did not show any statistical significance after allowing for confounding factors. This could be due to the insufficient sample size in that subgroup as indicated by the wide range of the 95% CI.

The associations of risk for oral cancer in smokers who also have genetic polymorphism have also been investigated. Some studies found that the polymorphism in phase 1 (GSTs) and phase 2 (CYP) may contribute to the risk of oral cancer (Katoh et al., 1999; Park et al., 2000; Kietthubthew et al., 2001; Buch et al., 2002), whilst other studies fail to find any association (Sharma et al., 2006; Hung, et al. 1997; Kietthubthew et al., 2006; Cha et al., 2007; Park et al., 1997; Olshan et al., 2000; Deakin et al., 1996; Worral et al., 1998; Drummond et al., 2004). The latter findings are similar to the findings of this study. The risk of oral cancer in smokers remained insignificant after adjusting for GSTM1, GSTT1 and CYP1A1 polymorphisms. The equal proportion of GSTs and CYP1A1 polymorphism and the fact that the majority of subjects are smokers among cases and controls may have some influence on the result in this study.

6.4.2 Alcohol habits and risk of oral cancer

In regards to other habits associated with oral cancer, it is well documented that alcohol consumption is one of the important risk factors for oral cancer, particularly in association with tobacco. It accounts for as much as 75% of all oral cancers in western and European countries (La Vecchia et al., 2004). The combination of alcohol and tobacco may result in a more synergized effect that would increase the risk of oral cancer. It should be noted that a study by Jaber et al. (1998) found that alcohol alone did not increase the risk of premalignant

lesions whilst another study showed a lack of clear experimental evidence for pure ethanol to be considered a carcinogen (Wright & Ogden, 1998) as the compound does not appear to react with DNA in mammalian tissue (IARC, 1988). However, epidemiologic studies on risk factors and oral cancer from many countries found an increased risk of subjects who practice alcohol drinking habit as well as with combination with smoking habit (Kabat and Wynder, 1989; Ogden and Wright, 1998; Altieri, 2004; Schantz and Yu, 2002; La Vecchia et al., 2004). The variation of risk estimation of each study is partly or largely due to different levels and/or sociocultural correlate of drinking patterns in various populations (La Vecchia et al., 1997).

The possible mechanism of the role of alcohol in developing oral cancers is due to the present of ethanol and as well as other carcinogens such as nitrosamine. Ethanol may increase the penetration of carcinogens (such as PAHs from tobacco product) by increasing the permeability of the oral mucosa (Du et al, 2000; Howie et al., 2001; Ogden, 2005). The amount of alcohol consumed varies in accordance to countries. In the United Kingdom, safe levels for drinking of alcohol equate to not more than 21 units per week for men and 14 units for women. Each unit contains 8 grams of alcohol (Odgen, 2005). It is known that some beers and whisky beverages also contain specific impurities or contaminants which can be carcinogenic. N-nitrosodiethylamine is present in some alcohol beverages. Eventually, the risk of oral cancer is likely to be due to a combination of influences both local (such as direct effect on cell membranes, alteration in mucosa permeability, variation in tissue distribution and type of enzyme involved in alcohol metabolism) and systemic such as nutritional

deficiencies, immunological deficiencies and disturbed liver function (Ogden and Wright, 1998).

The findings in this study showed that alcohol drinking was practiced among a very small number of subjects. It was accounted for by 13.6% among cases and 9.3% among controls. Furthermore, in term of risk for oral cancer due to alcohol consumption, studies done in many countries have reported a variety of odds ratios. Some studies found that consuming beer increases the risk ranging from 4-8 times; hard liquor reached 5-20 folds, whereas wine was lower (2-4 times) and that the risk of oral cancer is more likely to increase with increasing number of glasses per week (Blott et al., 1992; Gronbaek et al., 1998; Altieri, 2004; Odgen and Wright; 1998; Kabat and Wynder, 1989; Franceschi et al., 1992).

In this study, drinkers had increased risks of oral cancer with OR 2.58 (95%CI 0.79-8.57) and OR 1.11 (95%CI 0.29-4.25) respectively compared to non drinkers. However their differences were not statistically significant. The amount and duration of alcohol drinking also does not increase the risk for cancer. Similarly, the types of alcohol consumed (beer, and whisky) do not increase the risk with the exception of wine where the risk increased by 11-times (OR 11.71, 95%CI 1.39-98.77). The fact that only a few subjects consume alcohol probably affects the statistical value of the data. Though cigarette smoking is socially acceptable in Indonesia; this is not the case for alcohol use. Indonesia is predominantly a Muslim country and drinking alcohol is strictly forbidden. Alcohol use is considered to be much more (social) deviant behavior than smoking. There is also the possibility of underreporting of the consumption

of alcohol because of this. The very small number of subjects who reported to have consumed alcohol has made the analysis meaningless.

6.4.3 Betel quid chewing habits and risk of oral cancer

Betel quid chewing with or without the inclusion of tobacco has long been identified as a major risk factor for oral cancer in many countries such as Pakistan, India, Sri Lanka, Bangladesh, Thailand, Cambodia, Malaysia, China, Papua New Guinea, and some areas in Indonesia (Gupta and Ray, 2004). In Indonesia, previously because of its ancient history, betel quid chewing was socially acceptable among the elderly including women. However the trend of betel quid chewing habit now is being substituted by smoking habit and is mainly confined to the older generation since the early to mid 1900s in many rural areas. Furthermore, especially in the capital city of Jakarta, the prevalence of betel quid chewing has reduced significantly even among elderly people. The habit of betel quid chewing in Indonesia is similar to the countries mentioned earlier, except Taiwan. Betel quid with ripe areca nut and slaked lime is chewed first and then a large wad of finely cut tobacco is used to clean the teeth. It is then finally kept in the mouth for sometimes. The other way is by preparing the areca nut and slaked lime wrapped in betel leaf with additions of tobacco and sweeteners or condiments. In contrast, in Taiwan, betel quid chewers more often use the unripe areca nut with betel inflorescence or betel leaves and Yang et al. (1996) and others has reported increasing betel quid usage among adolescents in Taiwan. The prevalence is also high among aborigines (42.1%) and the habit starts in childhood around 12 years (Lu et al., 1993).

This study found that betel quid chewing habit was practiced among a very small number of subjects (9.2%). Among cases, only 7.4% of subjects had betel quid chewing habit. In terms of risk of oral cancer, after allowing for smoking, alcohol and diet pattern, there was a six-time higher risk of oral cancer among those who are chewer of betel quid. The combination of betel leaves, slaked lime, tobacco and areca nut (called complete combination) was the only preferred (9.3%) combination among subjects. No other preferences of betel quid compositions were noted in this study. Number of quid 1-10 quids/day also showed a six-time increased risk. The adjusted risk for oral cancer based on the preferred combination of betel quid (betel leaf, tobacco, areca nut, lime) in this study was almost five-fold higher compared to non chewers after allowing for smoking habit. In fact, all the betel quid chewers in this study were also tobacco smokers (*kretek* type). This supports the notion that areca nut and tobacco synergistically affect the mucosal tissue and thus lead to cancer. This is also supported by IARC (2004) which reported that betel quid containing areca nut with or without tobacco is carcinogenic. Other studies (Yang et al., 2005; Shah and Sharma, 1998; Lee et al., 2003) have shown that areca/betel quid chewing with or without tobacco served as important risk factors in developing premalignant lesions as well as oral cancer.

Evidence from epidemiological and experimental studies have shown that even in the absence of tobacco or lime, the areca nut in the betel quid has a potentially harmful effect on the oral cavity (Reichart and Philipsen, 2005). The IARC report (2004) has classified betel quid without tobacco as a Group 1 carcinogen, with increased risks observed for oral cancer. Areca nut has been classified as a Group 1 carcinogen, based on strong association and plausible mechanism for

carcinogenic action in vivo. It was also based on the findings that areca nut causes oral submucous fibrosis. The areca nut-specific nitrosamines, which cause tumor in animals, have been detected in the saliva of betel quid chewers (IARC, 2004). Areca nut contains potent cholinergic muscarinic alkaloids, notably arecoline and guvacoline, with a wide range of parasympathetic-omimetic effects. It promotes salivation and the passage of wind through the gut. It raises blood pressure and pulse rate and elicit a degree of euphoria by virtue of their GABA receptor inhibitory properties which contribute to dependence and habituation (Johnson, 2003b). Besides that, slaked lime (aqueous calcium hydroxide paste) is an essential ingredient in betel quid chewing practiced in some countries. This may lower pH and accelerate release of alkaloids from both tobacco and nut, with enhanced pharmacological effect. The used of tobacco in conjunction with areca nut results in exposure to tobacco related nitroso-compound, but not polycyclic aromatic hydrocarbons, which are the products from combustion. In addition, cancer related to betel quid chewing without tobacco, the exposure related to alkaloids contains in areca nut (arecoline, arecaidine and guvacoline) as well as the slaked lime which may cause habituation.

6.4.4 Genetic polymorphisms and risk for oral cancer

Molecular epidemiological studies have shown varying evidence that individual susceptibility to cancer is mediated by both genetic and environmental factors. The inherited differences in the effectiveness of detoxification or activation of carcinogens play a crucial role in host susceptibility. Phase I and phase II detoxification play important roles in preventing cells from damage. The cytochrome P450 (CYP1A1) phase 1 and the Gluthathion S-Transefrase (GSTs)

phase II are family of enzymes involved in metabolism of many environmental agents, including tobacco and alcohol, and play a role as cancer susceptibility factors (Chen and Hunter, 2005). However, polymorphism of GSTM1, GSTT1 and CYP1A1 genes and their effects on oral cancer studied in many countries world wide for the last thirty years have displayed varying result.

Meta-analysis data on ethnic differences in the baseline frequencies of GSTM1 null among control subjects have reported variations between 24 to 57.7% in Asian, 46.6 to 53.8% in Europeans and 15.9 to 57.8% in Americans (Ye et al., 2004). So far there are no studies on the prevalence of these genotypes in oral cancer that have been done in Indonesia. This study found that GTSM1 was overrepresented in cases compared to controls (60.5% vs. 55.5%). Among controls, the prevalence of GSTM1 null was within the range as in Asian, European and American population although it appears in the higher range.

However, in terms of risk, contrasting results were observed between risk of GSTM1 null from pooled-analysis done by Hashibe et al.(2003) from Asian, European and American countries (OR 1.53, 95% CI 1.19-1.97) and the findings of this study (OR 1.19, 95%CI 0.70-2.02). This study found that GSTM1 null genotype did not increase the risk of developing oral cancer in the Jakarta population. The risk of oral cancer remained statistically insignificant after allowing for the influence of the main risk factor in this study (smoking status). This finding is supported by many studies in several countries such as by Sharma (2006) in India, Hung, et al. (1997) in Taiwan, Srelekha (2001) in India, Cha (2007) in Korea, Park et al. (1997; 2000) and Olshan et al. (2000) in USA, Deakin et al. (1996) and Worral et al. (1998) in UK and in some other countries

where the head and neck or larynx cancers have also been included (Mathias et al., 1998; Jourenkova et al., 1999). In contrast, there are studies that did not support these findings. Such studies have been reported for the Japanese population (Sato et al., 1999; Katoh et al.,1999; Kihara, 1997) where the null GSTM1 genotype increased the risk of oral cancer by 2.2 times, 2.6 times among Thai ethnicity (Kietthubthew et al., 2001) and 22.8 times among Indians (Nair et al., 1999), which estimated risk for oral leukoplakia and precancerous lesions.

For GSTT1, Hashibe et al (2003) also reported that the prevalence of GSTT1 null genotype in the control series varied from 11.1 to 52.8% in Asian, 13.9 to 52.3% in European and 10-80% in American. This study found that the prevalence of GSTT1 null genotype is within the range reported for Asians (41.4%) although it appears in the higher range. However no significant difference was observed between cases and controls ($\chi 2$=0.4118 Pr = 0.521). The ORs of GSTT1 null based on pooled analysis were 1.13 (0.97-1.32) in Whites, 0.88 (0.49-1.57) in African-Americans and 1.19 (0.87-1.63) in Asians. Again, similar result for pooled ORs was observed in this study. Apparently, genetic polymorphism of GSTT1 did not increase the risk of oral cancer in the Jakarta population (OR 1.19, 95% CI 0.72-2.05). This result is supported by eight out of fourteen studies in meta-analysis (Geisler and Olshan, 2001) which have reported the weak association between GSTT1 null genotype and oral cancer in different ethnic groups (Buch et al., 2002; Deakin et al., 1996; Sikdar et al., 2004; Kietthubthew et al., 2001; Sreelekha et al., 2001) and results form pooled analysis done by Hashibe et al. (2003).

In contrast to the numerous studies available for GST, relatively fewer studies exist on CYP1A1 polymorphism in oral cancer development. Most of the research in CYP was done on pharynx, larynx and lung cancers. In this study, even though the prevalence of CYP1A1 polymorphism was found to be slightly higher in controls than cases, however the difference was not statistically significant. Similarly, CYP1A1 polymorphism also did not increase the risk of oral cancer in the Jakarta population. The result is consistent with the study done for Caucasians among French, German and Dutch suffering from head and neck cancers (Lucan et al., 1996; Hahn et al., 2002; Oude Ophius et al., 1998) and meta analysis from 31 published case-control study as well as pooled analysis from 9 published and 2 unpublished case control study done by Hashibe et al. (2003). This findings is in contrast to the results obtained from studies among Japanese suffering from oral cancer done by Sato et al. (1999) and Sreelekha et al. (2001), and study by Morita et al. (1999) in head and cancer population, where the CYP1A1 polymorphisms enhanced the risk of oral cancer.

Additionally, a study in Korea recently reported an increased risk of oral cancer when the GSTM1 and GSTT1 null genotypes are present together (Cha et al., 2007). The Korean smokers also showed that the combination of GSTM1 null and polymorphism of CYP1A1 valine/valine also significantly increased the risk of oral cancer in smokers. In contrast, the present study found that the combination of GSTM1, GSTT1 null genotypes and CYP1A1 polymorphism (isoleusin/valine and valine/valine) remained insignificant as risk factors to oral cancer, even after allowing for smoking status.

Conflicting data on the association between polymorphism of GSTM1, GSTT1 and CYP1A1 and oral cancer may arise from many sources. As enumerated by Marques et al. (2006), the problems can be encountered from differences in the distribution of polymorphic genotype among ethnic and geographical groups worldwide, as well as the methodological issues. Failure to properly sample cases and controls for hospital based studies can lead to bias of the gene-environment interactions if the controls do not reflect the exposure and/or genotype distributions of the source population.

Different methods in matching of the control may also result in bias. Matching is often utilized to increase the efficiency of statistical adjustment of confounding factors. The selection bias and residual confounding may be introduced when matching factors are not accounted for in analysis (Geisler and Olshan, 2001). Besides that the distinct role of enzymes in different tissues and cancers is another possibility yielding a different feature of genotypes. Thus, the ethnic differences in allelic frequency of GSTM1 and GSTT1 polymorphism and the differences in environment and lifestyle risk factors may also lead to discrepancy. When considering the latter, the types of tobacco used in cigarettes in Jakarta population (which comprised mainly of *kretek*) may have significantly different mixtures from various countries and thus may give different mechanism in influencing the interaction with drug metabolizing enzymes in the body. This may be attributed to the fact that study groups were taken from different ethnic populations and to difference in exposure of different carcinogens in different populations (Sharma et al., 2006).

6.4.5 Dietary habits and risk of oral cancer

An association between diet and oral cancer has long been suggested. Over the past three decades, several analytical epidemiological studies have been published on the possible dietary correlates of oral cancer. Earlier work found little association with any specific food, nutrient, or method of food preparation (Graham et al., 1977). The most consistent findings in diet as determinant of cancer risk is the association between consumption of vegetables and fruit in reducing risk of several cancers. About 80% of these studies found a significant protective effect of overall consumption of vegetables and/or fruit or at least of some types of vegetables and fruits (Grosvenor and Smolin. 2002; Cappuccio et al., 2003; Voorrips et al., 2000; Franceschi et al., 1999; 2000; Soller et al., 2001).

The complexity of the human diet presents a challenge as well as difficulty in interpreting the correlation between diet and oral cancer. Diet has traditionally been studied in terms of nutrition. However, food may contain other chemical compounds of which some are well-known, some still poorly characterized and others completely unknown and which at present cannot be measured. Furthermore, the diversity of food combinations may lead to competition, antagonism or alteration in nutrient bioavailability. From an epidemiological viewpoint, diet represents a complex set of highly correlated exposures. Thus, a real relationship between a food group and a disease may incorrectly be attributed to a single component, because of the multicolinearity that exists between nutrients and foods (Gordon et al., 1984; Willet, 2000).

In addition to the relative scarcity of studies on diet and oral cancer especially in Asia which differ from the dietary pattern in Europe and America where many research on diet and cancer are conducted, an even smaller number of studies have analyzed consumption patterns. Little is known about the association between dietary patterns and risk of oral cancer. This is due to the issue of diet being very complex due to the effect of not only one food item but also their interactions as several food types.

In this study the grouping of food was based on the similarity of nutrients in terms of their major components. The carbohydrate group consisted of rice, bread, noodle, sweet potato, cassava, sago, and corn where the major nutrient is refined as carbohydrate. The other group such as fast food also consisted of carbohydrate, however the grouping here was also based on the processing of the food which is characterized by the use of high temperature and inclusion of chemicals.

One way of dealing with the complexity of inter correlations between foods is the use of pattern analysis (Gordon et al., 1984; Randall et al., 1990; Trichopoulos, 2001), as has been done in this study. This approach uses correlations between food and nutrient intake to describe a general diet pattern that may be related to the risk of a disease at a later stage. This approach is of particular value if the effect of the diet is not mediated by one or two specific nutrients, but by nutrients that perhaps operate interactively. In the present study, the dietary patterns of participating subjects were identified using principal component analysis and these patterns were used for estimating the risk of oral cancer. The purpose is to identify the structure in the data matrix, by

summarizing and reducing data in order to supply a synthetic measurement of the diet or in other words to transform a large set of correlated variables into a smaller set of non-correlated variables that are called principal component. Factor analysis derives dimensions that, when interpreted and understood, describe the data in terms of much smaller number of items than do the individual variables (Happner and Happner, 2004).

This multivariate method may also represent an alternative approach to the evaluation of individual nutrients, since the identification of patterns allow one to examine the effect of the diet as a whole and to describe associations with disease beyond those described for single nutrients or foods. The patterns identified may be used as co-variables in order to determine whether the effect of specific nutrient is independent of the dietary pattern. Moreover, it is known that individuals consume nutrients based on their food choices, which are influenced by a variety of cultural, social and demographic factors.

In this study, the pattern identified as “preferred food” characterized by the consumption of fast food, cooked and raw vegetables, fermented food, seafood, canned food, and snack high in fat and sugar, was associated with doubling of risk of oral cancer. According to adjusted risk estimate, it was found that subjects with scores in the highest tertile of the “preferred food” pattern had greater risk of oral cancer than subjects with lower scores. This finding was consistent with many studies which found that fast food, fermented, canned food and meals that contain high fat and sugar may increase the risk of cancers and other disease such as hypertension and diabetes (Stewart and Kleihues, 2003). Fast food (chips, hamburger, pizza, ready made spices and especially instant

noodle) represented the greatest communalities in this study, which mean that this food type has much in common with other variables taken as a group and loaded in component which is labeled “preferred food”.

A plausible explanation of the contribution of these foods to oral cancer is that for fast food, which is mainly composed of high levels of fat can produce PAHs during curing (food preparation) using high temperature, and the PAHs found in the food have been shown to cause cancer in laboratory animals (Grosvenor and Smolin, 2002). In addition, the fermented, canned food or processed food may increase the risk of cancer (Stewart and Kleihues, 2003). The World Cancer Report reported that the consumption of salted food as well as preserved and canned food (rich in nitrate performed N-nitroso compounds) is associated with cancer especially gastric cancer. Several biological mechanisms also have been proposed to explain the association between Chinese-style salted fish and nasopharyngeal cancer, including partial fermentation and nitrosamine formation (Steward and Kleihues, 2003).

In contrast to studies carried out by Franceschi et al. (1991), Zeng et al. (1993), and Soler et al. (2001), the present study found that vegetables (cooked and raw) increased the risk of oral cancer. The mechanism for this finding is not clear. Furthermore, factor analysis indicates that single factor alone may not account for disease causation or prevention as other factors in the same component will interact interchangeably. However if it is seen from the common style of cooking vegetables in Indonesia, there are some plausible reasons for this observation. Astawan (2004) in his study found that there are four common styles of cooking vegetables in Indonesia. First is the habit of washing the

vegetables after cutting, which may lead to the wider surface area of vegetables being exposed to air and water and resulting in depletion of some mineral and vitamins (especially C and B) from the vegetables. Second is the habit of cooking the vegetables with an abundance of water which results in dissolving the majority of vitamins, minerals and amino acids in the soup and the remaining soup left uneaten. The third habit is to boil the water together with the vegetables to just before the water temperature reaches the boiling point; which may also deplete some parts of active constituent of vegetables. The fourth habit is to overcook the vegetables as well as reheating them many times. All the above styles may contribute to decreasing the quality of cooked vegetables in Indonesia and if they interact with other risk factors may increase the risk of oral cancer. The habit practiced in cooking vegetables in Indonesia mentioned above may deplete or reduce the active constituent (vitamins, minerals and acid amino) of vegetables which is beneficial in preventing cancers (Grosvenor and Smolin, 2002).

The second pattern called “combination” characterized by meat, dairy product and fruit showed inverse relationship to oral cancer. Fruit and vegetables have been consistently proven as a protective food type in many cancers. In regards to oral cancer, this finding is supported by studies done by McLaughlin et al. (1988); Zeng et al. (1993); Franceschi (1991); Franceschi et al., (1999); and Soler et al. (2001), who found the strongest protective effects derived from citrus fruit and others which are likelier to be eaten raw, thus pointing to a mechanical cleansing effect of raw fruit and vegetables on the oral cavity and/or to the beneficial effect of some temperature-sensitive substance(s), such as vitamin C.

The fact that meat and dairy product have been found to have a protective effect in this study is in contrast to some studies. Studies conducted in European countries found meat, cheese and milk increased the risk of oral cancer (Franceschi et al., 1999; McLaughlin et al., 1988). However, a study conducted by Franceschi et al. (1992) found that major sources of protein and fats in Italian diet (milk, eggs, oil and cheese) and some nonalcoholic beverages did not seem to have a significant influence on development of oral cancer. Similarly, Zeng et al. (1993) did a study in Beijing, and found that protein and fat intake are related inversely with oral cancer risk. Drinking milk showed a moderate inverse association with oral cancer risk with marginally significant OR of 0.5 (95%CI 0.2-1.0). One possibility for the differing finding between this and other European studies is that the food preparation in European food might be different from Asian, especially Indonesian. The most common meat preparation in this study was boiled (such as *soup, gulai and rendang*). Whereas majority of meat preparation in European countries are steak type which needs to be grilled, baked or roasted and sometimes deep fried. As mentioned above the PAHs are carcinogenic and found in greater amount in this type of food. Grilled meats are high in PAHs because they are formed when fat drips onto the flame below. The broiled and deep fried meats also contain another hazard as it contains heterocyclic amines (HAs) such as benzopyrene which is formed from the burning of amino acids and other substances in the meat under very high temperature (Grosvenor and Smolin, 2002). This theory is supported by studies which found the positive associations of oral cancer with intake of nitrite-rich meat (McLaughlin et al., 1988) and pork product (Levi et al., 1998), salted meat (Zeng et al., 1993) and charchoal-grilled meat (Franco et al., 1989). The second possibility why meat and dairy product may have protective effect is related to

the basic needs of the body for essential amino acid in cell building and defense (immune) mechanism (Grosvenor and Smolin, 2002).

The highest tertile scores of the third food pattern categorized as “chemical related” food significantly increased the risk of oral cancer. This third component was loaded by processed food and MSG. The chemical contained in this food group has been emphasized as carcinogenic agent to cancer. However, this still need further investigation as some review stated that monosodium glutamate (MSG) is the amino acid which can be metabolized automatically by the body (Grosvenor and Smolin, 2002)..

The fourth component which also increased the risk of oral cancer in this study is categorized under “traditional”. It is characterized by all type of drinks (soft drink, canned drink, coffee and tea) and staples food (Indonesia majority rice) which produce high calories. This finding is also supported by Franceschi et al. (1999) who found an association between drinks and oral cavity and pharynx cancers. The OR of highest tertile of daily intake of soft drink was 1.6 (95%CI 1.3-2.2) compared to the lowest intake. Carbohydrate intake has also been shown to moderately increase the risk of oral cancer in the study conducted in Beijing by Zeng et al. (1993).

Since dietary patterns were extracted from data obtained within the study population, the results cannot be generalized for the Indonesian populations with different dietary habits. However, as studies in dietary pattern in Indonesia is scarce, especially using principal component (factor) analysis, the pattern observed in this study provides an indication of the common food consumed. Based on these findings, and as a start for the Indonesian population, dietary

guides are issued with emphasis on foods and on overall dietary patterns which are health promoting or damaging to the health of the population.

In this study, the food items were grouped by the similarity in terms of composition and nutrient value, based on previous studies (Franceschi et al., 1992; Franceschi et al., 1999; Zeng et al., 1993; Marchioni et al., 2007). Futhermore the four retained factors explain more than half of the total variance. According to Schulze at al. (2001), if the patterns fail to explain much of the variance in the food intake as a whole, it is possible that these patterns would not explain much of the variance in a single food or nutrient either, thus limiting their use in nutritional epidemiology.

Dietary pattern results from factor analysis are yielding the pattern of diet retained from certain population and are not appropriate enough to see the relationship between nutrients and disease through only knowing the dietary pattern analysis since this analysis is not specific for such purposes. Pattern analysis may be useful when the traditional approach, which focused on nutrients, identifies only a small number of specific associations with the disease. Further research is suggested on the effects of micro and macro nutrient composition of food as well as the calories intake and in establishing its link to oral cancer, so that it will be possible to develop nutritional intervention based on these patterns in Indonesia.