Chapter 1

Introduction

1.1 Problem statement

Oral cancer is a serious public health problem in the world with over 390,000 new cases reported annually worldwide. Of these figures, two-thirds of which occur in developing countries. This malignancy is responsible for some 200,000 deaths each year (Sankaranarayanan, 2003). Most of the oral cancers are asymptomatic at the early stages. Because the lesion may be a painless small ulcer, many individuals are not aware of the importance to seek early treatment. A good cure rate is possible if oral cancer is detected at the early stage. Sadly, over 60% of patients usually present with advanced stage of oral cancer (Sankaranarayanan, 2003). Approximately 70% of those who presented at the late stage had nodal metastases. It is also important to note that a significant percentage of patients with oral cancer tended to develop a second primary tumor although initially the disease was cured (Warnakulasuriya, 2002). Till date, the management of oral cancer is rather complex. Currently, the most effective remedy for treating oral cancer is through surgery combined with radiotherapy. Despite the fact that several treatment improvements have been achieved in surgical techniques, radiation therapy protocols, and chemotherapeutic regimes (Cooper *et al*., 2004), the overall 5-year survival rate for this disease remains at 50% and has not significantly improved in the past few decades (Johnson, 2003). Meanwhile the surviving patients may be left with severe aesthetic or functional morbidity (Tsai *et al*., 2004), which could lead to a reduction in quality of life of the patient (Sankaranarayanan, 2003).

It has been recognized worldwide that tobacco smoking, alcohol consumption and betel-quid chewing are the three main risk factors found to be associated with oral cancer (Johnson, 2003). In fact, many studies had shown that synergistic effects of smoking and alcohol consumption have increased the risk of oral cancer (Boffetta, 2003a). Although the distinct risk factors for oral cancer are well-recognized, little is known about the molecular mechanisms responsible for this malignancy. The development of oral cancer proceeds through several molecular genetic events, with cumulative damage in specific genes initiated through loss of genomic integrity, often after long-term exposure to environmental risk factors, particularly tobacco and/or alcohol (Rai *et al*., 2004). Hence it is of great interest to know that the polymorphic genotypes which code for tobacco carcinogen-metabolizing enzymes (for instance, glutathione s-transferase – GST) could play an important role in oral cancer susceptibility (Park *et al*., 1999).

Besides the role of genetic susceptibility, there are many documented literature which focused on the studies of diet and nutrition as the risk factor for oral cancer. There was clear evidence that consumption of vegetables and fruits is associated with reduced risk of oral cancer (Pavia, 2006). In general, these studies focused on the protective effect of some of the mirconutrients such as Vitamins A, C, E, β-carotene and selenium which has been attributed to their antioxidant activities (Zain, 2001). Lately, considerable interest was also generated on the phytochemicals which have health-promoting compounds found predominantly in plant foods. One such phytochemical known as isothiocyanate (ITC), found abundantly in cruciferous vegetables and its important role as anti-carcinogens has yet to be fully understood.

1.2 Significance of study

Oral cancer is a tobacco-related disease that represents a significant problem based upon its high incidence in many parts of the world, the poor survival rates associated with this type of malignancy, and the severe functional and cosmetic defects accompanying the treatment of this disease.

The metabolic products from environmental exposure such as tobacco smoke, alcohol, betel quid and even diet can induce direct deoxyribonucleic acid (DNA) mutations and increase the production of reactive oxygen species (ROS). These free radicals can lead to DNA damage and lipid peroxidation which could harm our body. The excess burden of free radicals caused by cigarette smoke may be balanced, in part, by the intake of antioxidants in plant foods (Gaudet *et al*., 2004). However, several enzymatic systems, including phase I and phase II enzymes are also involved in metabolism of environmental agents. The coordinated expression and regulation of the enzymes within these two metabolic phases have been shown to modulate cancer risk (Sato *et al*., 1999; Sreelekha *et al*., 2001). Phase I enzymes metabolism are catalyzed by cytochrome P450 (CYP) that oxidizes the compounds into reactive electrophilic metabolites followed by Phase II conjugating enzymes such as glutathione s-transferases (GSTs) involved in detoxification of chemicals. Individual variation in enzymes activating or detoxifying carcinogens and other xenobiotics have subsequently been related to the discovery of genetic polymorphisms for these genes and may contribute to the differing cancer development potential in different individuals (Tsukino *et al*., 2004).

The absence of *GSTM1* and *GSTT1* and the polymorphism of *GSTP1* results in decreased or lower activity of detoxification of carcinogens. These metabolic deficiencies may predispose individuals to the development of smoking-related tumors, such as oral and lung cancers. Several studies on reliable genetic markers for individual susceptibility to oral cancer have yet to be confirmed. Two preliminary studies examining *GSTM1* null genotype as a determinant for oral cancer risk have been described, but these studies have produced conflicting results concerning the importance of this genotype in oral cancer susceptibility (Trizna *et al*., 1995; Cha *et al*., 2007). A link between the susceptibility to oral cancer and *GSTT1* null genotype is however, supported by relatively high incidence of oral cancer patients exhibiting detectable levels of GSTT1 protein (Jourenkova-Mironova *et al*., 1999; Buch *et al*., 2002). On the other hand, many studies revealed that there was no association between the *GSTT1* null genotype and the risk of oral cancer (Olshan *et al*., 2000; Kietthubthew *et al*., 2001; Sreelekha *et al*., 2001; Capoluongo *et al*., 2006; Sugimura *et al*., 2006). As for the *GSTP1* polymorphism genotype and oral cancer risk, there were also a few studies reported with inconsistent results. Jourenkova-Mironova *et al*. (1999), Olshan *et al*. (2000) and Cho *et al*. (2006) found that there were no statistical significant correlation between the *GSTP1* polymorphism genotype and risk of oral cancer. However, Morita *et al*. (1999) found that polymorphism of *GSTP1* may confer a slight increase in risk for oral cancer. Overall, there are only a few studies on *GSTM1, GSTT1, GSTP1* polymorphism and oral cancer risk has been reported. Occasionally, polymorphisms of the *GSTM1, GSTT1* and *GSTP1* genotypes have been associated with oral cancer but most studies to date had reported no association.

Knowledge of the specific genetic polymorphism conferring this susceptibility should provide more power for the detection and characterization of environmental risk

factors through stratification of the sample according to underlying genetic make-up. Likewise, there may be environmental factors that are associated with cancer in all individuals, but with a much stronger effect in individuals who have a reduced capacity to metabolize the relevant carcinogens (Goldgar, 2003). Many researchers have reported susceptibility related to inherited capacity to metabolize carcinogens or pro-carcinogens. This especially appears to involve polymorphisms in the *GSTs* genes. Identification of inter-individual cancer susceptibility is an important factor in cancer prevention and early detection (Morita *et al*., 1999).

Strong efforts have been made to identify active compounds in cruciferous vegetable and to understand the molecular mechanisms which cause their protective effects. Overall, the most important principles of chemoprevention by ITC are induction of phase II enzymes and inhibition of phase I enzymes. Result from a study show an inverse relationship between high intakes of dietary ITC and lung cancer risk among the *GSTM1* non-null genotype (Wang *et al*., 2004). With regards to colorectal cancer, however, a study conducted by Seow *et al*. (2002) recorded that for those with both polymorphism of *GSTM1* and *GSTT1*, there was a 57% reduction in risk among high versus low dietary ITC intake. Similar to the previous findings, the protective effect of ITC was also seen primarily among individuals with polymorphism of both *GSTM1* and *GSTT1* and lung cancer risk (London *et al*., 2000). To date, there was no study done on the dietary ITC intake in relation to oral cancer risk.

No reports to date have explored the influence of dietary ITC and *GSTM1*, *GSTT1* and *GSTP1* polymorphisms on the risk of oral cancer. Only one report by Gaudet *et al*., (2004) evaluated the interactions between fruits and vegetables and *GSTM1* and *GSTT1*

polymorphism on the incidence of head and neck squamous cell carcinoma (HNSCC) using data from a case-control study. However, their results failed to support clearly the hypothesized role of an interaction between plant food and *GSTM1* and *GSTT1* polymorphism on the risk of HNSCC.

In the Malaysian scene, so far there is no literature on the *GSTM1, GSTT1* and *GSTP1* polymorphism and the risk of oral cancer. The only available information is from a preliminary study conducted by Zain *et al*. (2006) on genetic polymorphism of *CYP1A1, GSTM1* and *GSTT1* genes in Malaysians (IADR, 2006). This preliminary study showed a lack of evidence to support the association between incidence of oral cancer and genetic polymorphism of *GSTM1, GSTT1* and *CYP1A1*. Despite many suggestive evidences that consumption of cruciferous vegetables may be associated with a reduced cancer risk (Jiao *et al*., 1998), no studies have been carried out in the Malaysian population to define the role of dietary ITC intake associated with *GSTs* polymorphisms and the risk of oral cancer. The only study done in this region evaluating similar vegetable resources was a health study on Singaporean Chinese on dietary ITC intake, *GSTs* polymorphisms and colorectal cancer risk. In fact, due to growing interest in the potential protective effects of dietary ITC in human cancer development, a database of glucosinolate and total ITC contents in various cruciferous vegetables from specific geographic regions is essential for assessing dietary ITC exposure in epidemiologic study (Jiao *et al*., 1998).

Perhaps it is time that research is focused on the consumption of certain group of vegetables rather than a broader aspect of all vegetables. Certainly this benefit may vary with different types and preparations of different groups of vegetables. A proven

association between the risk of oral cancer and the consumption of fruits and vegetables would give considerable public health and nutritional implication.

Therefore, this study was carried out to explore the effect of dietary ITC and *GSTs* polymorphisms on oral cancer risk. This may pave the way to better understand the causes of oral cancer and thus prevent the ailment by improving one's diet. Hence, in order to examine the association between dietary ITCs, *GSTs* polymorphisms and dietary ITCs-*GSTs* polymorphisms interaction and OSCC risk, this study will focus on the following objectives and hypotheses.

1.3 Aim

The aim of this study is to examine the association between dietary isothiocyanates (ITCs), *GSTs* polymorphisms, dietary ITC- *GSTs* polymorphism interaction and oral cancer risk.

1.3.1 Objectives

1. To determine the dietary ITC intake and its association with oral cancer risk.
2. To determine the *GSTM1, GSTT1* and *GSTP1* polymorphism and its association with oral cancer risk.
3. To determine the association between dietary ITC-*GSTs* polymorphisms interaction and oral cancer risk.

1.4 Hypothesis

The research or alternate hypotheses are:

i. There is an association between dietary ITC intake and oral cancer risk.

ii. There is an association between *GSTs* polymorphisms and oral cancer risk.

iii. There is an association between dietary ITC-*GSTs* polymorphism interaction and oral cancer risk.

The statistical or null hypotheses are:

i. There is no association between dietary ITC intake and oral cancer risk.

ii. There is no association between *GSTs* polymorphisms and oral cancer risk.

iii. There is no association between dietary ITC-*GSTs* polymorphisms interaction and oral cancer risk.