CHAPTER 1

INTRODUCTION

1.1 Introduction

Oral Cancer can occur at different intraoral sites including the lip, tongue, alveolus floor of the mouth, buccal mucosa and hard palate. It is the sixth most prevalent malignancy worldwide and the third most common cancer among the developing nations (Chiou et al., 2008). The incidence of oral squamous cell carcinoma (OSCC) is low in most western countries. However, in the Indian subcontinent and other parts of Asia, OSCC is considered as one of the most common forms of cancer (Moore et al., 2000).

China is one of the six high-risk areas worldwide for oral cancer. In China, the incidence of oral cancer has jumped from 81 per 1,000,000 population to 202 per 1,000,000 in the past few years. It is estimated that the mortality rate in China has reached around 10,000 cases annually due to oral cancer. As oral cancer is usually detected at the late stage, the survival rate of such patients has been reported to be only about 40-50% (Zhou et al., 2009). Oral cancer is also an important problem in India because of the widespread habit of betel quid chewing, and this habit plays an important role in the etiology of this disease (Nagpal et al., 2002). Some earlier studies have reported the dose relationship between the consumption of tobacco or alcohol or both together on oral cancer (Subaprya et al., 2002).

Tobacco contains 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N-nitrosonornicotine (NNN). These are tobacco-specific nitrosamines present in tobacco products and smoke. Both compounds are carcinogenic in laboratory animals which interact
with their metabolite activities and generating tumors. This is comparable to those observed in smokers (Peterson, 2010). Both normal metabolic activities and several other environmental factors like UV light and radiation can also cause damage to the DNA molecule, which could result in as many as 1 million individual molecular lesions per cell per day (Lodish et al., 2000). As a major defense against these damages to the DNA, cells poses the vital ability to identify and corrects the damaged DNA molecules, which is vital to the integrity of its genome and thus to its normal functioning and that of the organism. Thus, DNA repair helps to minimize cell killing, mutations, replication errors, persistence of DNA damage and genomic instability.

DNA repair refers to a collection of processes by which a cell identifies and corrects the damaged DNA molecules that encode its genome. Mutations and polymorphisms have been identified in many of the genes coding for DNA repair enzymes and tumor suppressor genes (Lunn et al., 1999). These changes are involved with certain proteins and are important for protecting the cells from abnormal growth and metastasis by repairing the damaged DNA before transcription and also for arresting the cell cycle to have enough time for repairing the pathways. One such important protein is the X-ray cross complementary group 1 protein (XRCC1) which plays an important role in the base excision repair (BER) pathway and interacts with DNA polymerase Beta (POLB), Poly ADP ribose Polymerase (PARP) and DNA ligase III (Zhang et al., 2006).

Earlier studies have suggested that XRCC1 protein has an important role in the etiology of smoke-related squamous cell carcinoma occurring in head and neck cancers (Olshan et al., 2002). It also has a breast cancer susceptibility gene product (BRCA) domain, which is the
characteristic of proteins involved in cycle checkpoint functions and this domain may be responsive to DNA damages (Mates et al., 2007). Shen et al. (1998) have reported five polymorphisms in the XRCC1 gene, where three of which occur at conserved sequences and resulted in amino acid substitutions. These three coding polymorphisms were detected at codons 194 (Arg-Trp), 280 (Arg-His), and 399 (Arg-Gln).

In the past many studies have been conducted to investigate the DNA repair genes in different kind of cancers such as XRCC1 (more genes). Indeed, genetic susceptibility on OSCC has frequently been reported for head and neck cancers (Sturgis et al., 1999; Sturgis et al., 2000; Tae et al., 2000; Shen et al., 2001; Spitz et al., 2001; Olshan et al., 2002; Shen et al., 2002; Sturgis et al., 2002 and Benhamou et al., 2004). Most of these investigations were extensively reported among the Caucasian populations, but only few studies have been conducted among the Asian populations until now (Tae et al., 2000; Hsieh et al., 2003; Kietthubthew et al., 2006). Ramachandran (2006) have reported that the presence of the polymorphic variant of XRCC1 codon 399 is associated with increased risk of oral cancer. However, Gal et al. (2005) reported that polymorphisms of the XRCC1 399 gene are associated with a decreased risk of OSCC. A study done by Tae et al. (2000) reported that there are possible association between XRCC1 polymorphism with Squamous Cell Carcinoma of the head and neck cancers and they suggested that this could be considered as a marker for genetic susceptibility in the Asian population. However, considering the paucity of information, further detailed studies are needed to validate the genetic effects of XRCC1 polymorphisms in the Asian population.
1.2  Aim, Objectives and Hypothesis

1.2.1  Aim

To investigate the distribution and role of XRCC1 Arg399Gln polymorphism in the development of oral squamous cell carcinoma among Malaysian population.

1.2.2  Specific Objectives

1. To determine the distribution of XRCC1 Arg399Gln genotypes in oral cancer (cases) and non-cancer (controls) patients.
2. To investigate the association between XRCC1 Arg399Gln genotypes and oral cancer risk.
3. To compare the distribution of XRCC1 Arg399Gln genotypes between different ethnic groups in Malaysia.

1.2.3  Null Hypothesis

1. There is no association between XRCC1 Arg399Gln genotypes and oral cancer risk.
2. There is no difference in distribution of XRCC1 Arg399Gln genotypes between different ethnic groups in Malaysia.
CHAPTER 2:

LITERATURE REVIEW

2.1. Epidemiology of Oral Cancer

To understand the cancer epidemiology, first the definitions of some basic words like incidence, prevalence and mortality must be understood. Incidence is frequency and the rate of occurrence of cancer among specific groups of population. People who have the specific disease among the population are considered as cases. These cases are considered as an absolute number of cases per year or as a rate per 100,000 persons per year. For cancer research, the word “case” is considered for a patient who has the average risk of developing cancer in one year (Parkin et al., 1999).

Prevalence describes the number of people who are still alive with the particular disease at a particular point of time. For cancer, there is no clear agreement on what is meant by “having” the disease. Some authors take “having cancer” as patients who have been diagnosed with cancer, even if it was diagnosed many years ago and also the case are cured (Parkin et al., 2005). Mortality on the other hand is the number of deaths occurring among particular cases and the mortality rate is the number of deaths per 100,000 persons per year.

Therefore mortality is considered to measure the average risk associated to the population dying from a specific cancer within a specified period (usually one year) (Parkin et al., 2005).
2.1. 1. Incidence and prevalence of oral cancer

Cancer is one of the most common causes of morbidity and mortality today. It is estimated that around 43% of cancer deaths are due to tobacco use, unhealthy diets, alcohol consumption, inactive lifestyles and infection. Low-income and disadvantaged groups are generally affected and are common with the ill effects of cancer. They have less access to health services; therefore they are more exposed to risk factors such as environmental carcinogens, alcohol, infectious agents and tobacco (Petersen, 2008). A recent meta-analysis of 41 studies has shown that low social economic status and poverty are significantly associated with an increased risk of oral cancer compared to other factors such as ethnicity (Conway et al., 2008).

In 2010, Warnakulasuriya reported in his review that oral cancer was considered as the sixth most common cancer in the world (Warnakulasuriya, 2010). According to this report, the annual estimated incidence was around 275,000 for oral and 130,000 for pharyngeal cancers excluding the nasopharynx, which were high among the South and Southeast Asians. According to a recent study, oral cancer is the third most common cancer after stomach and cervical cancer in the developing countries. An estimated 378,500 new cases of intra-oral cancer are diagnosed annually worldwide (Ayaz et al., 2011).

In the Indian subcontinent and in other parts of Asia, oral cancer remain as one of the most common forms of cancer (Moore et al., 2000). China has the highest number (2.2 million or 20.3 % of the world’s cases), where limited radiation facilities make it difficult to treat. Oral cancer rates are higher in men in South Asia (12.7 per 100,000) compared to women (8.3 per 100,000). According to Warnakulasuriya (2010) countries belonging to the
high risk category such as Sri Lanka, Pakistan and Taiwan, the oral cancer was the most common cancer among men. The sex difference is influenced by heavier risk habits such as tobacco and alcohol. High risk of oral cancer among Asians compared to other populations could be due to different lifestyles, especially the habit of chewing betel quid containing tobacco and/or areca nut.

Earlier studies have reported oral cavity as the most common site and accounts for about 40% of all cancers in India and Sri Lanka (Sankaranarayanan, 1990; Zakrzewska, 1999). India has always been known as a country with the highest incidence of oral cancer in the world with more than 100,000 cases reported every year. However, according to some recent reports, Sri Lanka and Pakistan are ranked as countries which have high cancer incidence. Among the Asian countries, Japan has the lowest incidence of oral cancer. According to a data from 10 population-based cancer registries in Japan during the year 2001, the rate of incidence was only 5.3 per 100,000 (Warnakulasuriya, 2010).

The second report of the National Cancer Registry (NCR) data revealed that a total of 21,464 cancer cases were diagnosed among Malaysians in the peninsular region during the year 2003 (Lim and Yahaya, 2004). In this report, oral cancers were reported separately as lip, mouth and tongue cancers. Among males, mouth and tongue cancers were ranked as 19th and 17th respectively of all cancers reported in Malaysia. Among females, mouth and tongue cancers were ranked 16th and 21th respectively of all cancers. When both mouth and tongue cancers were considered together, cancers of the oral cavity in the Malaysian population accounted for 2.5% of cancers among the male and 2% of cancers among
female making it the 12th commonest cancer among the men and 13th among the women (Lim, 2002).

Based on the Annual Report (1996) from the Ministry of Health, quoted by Zain (2001), lip and oral cancer were the third most common cancer causing deaths in government hospitals of Malaysia. In fact, this malignancy accounted for 7.1% of cancer deaths reported from the facilities of the Ministry of Health. Although the prevalence of oral cancer is low in Malaysia (0.04%) (Zain et al., 1997), about 60% of oral cancer lesions have been found to occur among the Indian ethnic group who comprise only about 8% of the total Malaysian population (Lim and Yahaya, 2003). According to Lim (2008) there was marked variations in the incidence of oral cancer among different ethnic groups of Malaysia. When the incidence was investigated according to specific ethnic group, it was found to be highest among the Indians. In the year 2003-2005, mouth cancer was ranked as the third most common cancer among Indian females while cancer of the tongue was ranked as the seventh most common among Indian males (Lim, 2008).

In some Asian countries like Thailand there is a decreased prevalence of oral cancer over the years. This is possibly because of the fact that risk habits such as betel quid chewing and smoking of traditional cigars are not so popular among the Thai population (Reichart et al., 2006). Oral cancers are reported most common among males. However, the male to female ratio has shifted from 6:1 in 1950 to 2:1 by 1997. The changing ratio is more because of the increased rate of smoking among women in the past three decades.

Cancer is also an age-related disease. Most cases of oral cancer in the United States are diagnosed in the sixth and seventh decades of life, with the highest prevalence noted in
patients over 65 years of age. However, studies have reported an increase in the incidence of oral cancer, particularly tongue cancer in young white males under the age of 40 (Shiboski et al., 2000). In the United States the number of women aged >65 years now exceeds the number of men aged >65 years by almost 50%. Though the number of affected white males in the United States has decreased from 1973 to 1996, but during the same time period an increase of cancer have been noted among the African-American males (Shiboski et al., 2000).

2.2. Clinical characteristics of oral cancer

Almost all oral cancers begin from the squamous cells covering the surfaces of the oral cavity. Therefore, oral cancer most commonly occurs on the lip, floor of mouth, tongue, hard palate, soft palate, gums and other areas of the oral cavity (Yuan et al., 2006). Different oral cancer sub-sites according to International Classification of Diseases (ICD 10 C00-C06) may be associated with different risk habits practiced by patients during their lifetime. Oral cancer in different sub-sites may have different behaviors leading to different prognoses. Oral cancer may present as white, red, ulcerated, exophytic, lumps, fissures or a combination of all these features (Zain et al., 2002).

2.2. 2. Histopathological characteristics of oral cancer

More than 90% of oral cancer is squamous cell carcinoma (SCC) (Johnson, 2001). SCC is a malignant epithelial neoplasm with squamous differentiations and the formation of keratin that may present with intercellular bridges. According to Pindborg et al., (1997), SCC can be categorized into different grades. The first grade is the well differentiated type showing keratinization with few mitotic structures and cellular pleomorphism. The second
grade is moderately differentiated and has less keratinization with more nuclear and cellular pleomorphism. The third grade is poorly differentiated and in this type, keratinization is rare and intercellular bridges are less than in the moderately differentiated type. The fourth grade is the undifferentiated carcinoma. This undifferentiated type is the carcinoma that does not show evidence of squamous differentiation.

Generally the well and moderately differentiated types are placed in the same group describing them as low grade, while the poorly differentiated and undifferentiated types are described as high grade. When a tumor shows different grades of differentiations, the higher grade is considered as the final grade (Pindborg et al., 1997).

2.3. Risk Factors of oral cancer

The most important etiological factors for oral cancer are tobacco and alcohol consumption, which if controlled could help in avoiding many tumors (Moreno-Lopez et al., 2000). Oral cancer is also strongly related to betel quid chewing. Betel quid chewing habits are not so common in most western countries, but in the Indian subcontinent and in other parts of Asia it remains one of the most common etiological factors for oral cancer development (Moore et al., 2000). In developed countries, the most important etiological factors are tobacco usage and excessive consumption of alcoholic beverages, where both of these factors act separately and synergistically. In these countries, the risk of oral cancer related to these two factors combined is estimated to be more than 80% (Rodriguez et al., 2004). Ethnicity also strongly influences the prevalence of cancer because of the social and cultural practices, as well as socioeconomic differences (Johnson, 2001). Effects of tobacco use and heavy alcohol consumption together explain the high occurrence of over 90% of
cases of head and neck cancer. Nevertheless, all forms of tobacco increase the risk of oral cancer (Johnson, 2001).

2.3.1. Tobacco

On a global view the use and abuse of tobacco products is the major cause of oral cancer (Johnson, 2001). Smoking is the leading cause of deaths, which first results from cancer disease. At least 15% of all cancers are estimated to be result of smoking (Parkin et al., 1994). It was estimated that 4.9 million people were using tobacco in the year 2000 and it is also estimated that by 2020 the figure will rise to 10 million per year with 70% of them being from developing countries (WHO, 2000). Many diseases such as heart disease, strokes, lung disease, lung cancer, oral cancer, gum disease and tooth loss are associated with tobacco use.

A case control study in Spain showed an increased risk of lip cancer when smokers have the habit of leaving the cigarette on the lip (Lopez et al., 2003). Patients with head and neck cancer who smoke were more likely to develop oral cancer that spread to their lymph nodes (Mansour et al., 2003). According to a meta-analysis study, on average a smoker have a three-fold increased risk of oral cancer (Gandini et al., 2008). Both dose and duration of smoking are associated with the risk of oral cancer, while termination of smoking leads to a fall in risk (Rodriguez et al., 2004).

After smoking cessation, the extra risk of oral cancer from smoking could almost disappear within 10 years (Dai et al., 2004). However, another study showed that it takes more than 20 years for the risk to reduce to that of never smokers (Malvezzi et al., 2008). All of the cells that are exposed to tobacco products sustain many changes. Thus, the entire
oral cavity, lungs and larynx are at risk for developing tumors. Patients do not just develop one tumor but may develop second or third lesions (Vaamonde et al., 2003).

In Malaysia, there are five million smokers over 15 years of age (Abu Bakar, 2006). The second National Health Morbidity Survey also reported that one in every four Malaysians is a smoker. Haniza et al (1999) showed that smoking is a popular habit which involved 27.9% of Malays, 19.2% of Chinese and 16.2% of Indians.

2.3.2. Betel-quid

In many countries of Asia and among migrated communities in Africa, Europe and North America, betel quid chewing is a common habit. It is accepted in many societies and is also popular among women. After caffeine, nicotine and alcohol, areca nut is the fourth psychoactive substance in the world. There are many ways and variety of ingredients for betel quid preparation in different countries (Gupta and Ray, 2004).

Quid is defined as a substance or mixtures of substances placed in the mouth of a person or chewed and remain in contact with the mucosa, usually containing one or both of the two basic ingredients, tobacco and/or areca nut, in raw or any manufactured or processed form. Betel quid is described as any quid (containing tobacco and/or areca nut) with betel leaf with or without other contents (Zain et al., 1999).

The quid can be divided into 3 basic categories (Zain et al., 1999):

1) Areca nut quid: Quid with areca nut but without tobacco products.

2) Tobacco quid: Quid with tobacco products but without areca nut.
3) Areca nut tobacco quid: Quid that has areca nut and tobacco products

Many populations in the world are using areca nut and this habit is endemic throughout the Indian subcontinent and large parts of South Asia (Gupta and Warnakulasuriya, 2002). The habit of chewing betel quid have been reported from many countries such as Pakistan, Sri Lanka, Bangladesh, Thailand, Cambodia, Malaysia, Indonesia, China, Papua New Guinea, several Pacific Islands and migrant populations in places like South Africa and Eastern Africa, United Kingdom, North America and Australia (Gupta and Warnakulasuriya, 2002). In fact the composition and method of chewing can vary widely from country to country. In Asia betel quid chewing is rooted from Asia’s social customs and the ingredients are easily accessible. Although in some countries like Thailand and Cambodia, these customs is losing popularity, the usage is increasing in other Asian countries. According to Asian health officials, many new betel quid users are adolescents and children (Srithavaj and Thaweboon, 2006).

According to the Taiwan government reports, about 10% of their population chews betel quid. The National Taiwan University Hospital in Taipei reported that from 1981 to 2000, a huge increase in oral cancer reports among Taiwanese men. This was significantly co-related to a seven-fold increase in areca nut production of the country (Parsell, 2005).

In Malaysia the habit of betel quid chewing is commonly found among certain ethnic groups, which are the Indians and the indigenous people of Sabah and Sarawak (Zain et al., 1997). Among the ethnic groups, betel quid chewing among Indians are most common where they use tobacco in the betel quid (Zain and Ghazali, 2001). According to a
study by Reichart (2006) betel quid chewing habits was observed more commonly among the younger generation and urban communities in Malaysia.

2.3.3. Alcohol

Alcohol is one of the very few examples of chemicals which have never been shown to cause cancer in experimental animals but which is nevertheless clearly involved in the causation of certain human cancers (Albert, 2007). Cancers of the mouth was investigated as early as 1956 by Wynder et al (1956) who found that the risk was directly related to the number of drinks, even after adjustment with smoking habit. Later studies in the USA (Graham et al., 1977) re-confirmed this finding. In another study in British Columbia, Canada, alcohol and smoking were found to be the major risk factors for oral cancer (Elwood et al., 2006).

For almost 50 years, alcohol drinking and tobacco together has been known as serious risk factors for oral cancer. One study showed doubling in the risk of oral cancers for people who drink 14 grams of alcohol/day (Castellsague, et al., 2004). Drinking alcohol in non-smokers is the most important oral cancer risk factor (Rodriguez et al., 2004). People who both drink and smoke have a much higher risk of oral cancer than those using only alcohol or tobacco (Blot, 1992).

Heavy drinkers and smokers have 38 times the risk of oral cancer compared to those who refrain from both products (Blot et al., 1988). The risk of oral cancer for those who drink alcohol is related to the dose of alcohol, even if it is not with tobacco smoking (Schantz and Yu, 2002; La vecchia et al., 2004). The risk of head and neck cancer is 5-10 times higher in heavy drinkers compared to others. The carcinogenic effect of alcohol
appears to be more in the oral cavity, pharynx and orophargus, but seems to be less effective in the larynx (Stewart and Kleihues, 2003).

According to the Ministry of Health Malaysia in the year 2006, the prevalence of alcohol drinking in the Malaysian population was around 21.1%. About 1.5 million drinkers were between 25-64 years old. Ethnically, only 0.4% of Muslim Malays between 25 and 64 years were current drinkers due to religious prohibition. About 24.2% of Chinese, 21.1% of Indian were active drinkers. Among sex, men were the most common users of alcohol in Malaysia (Tan et al., 2009).

2.3.4. Other risk factors

A. Human Papilloma Viruses (HPV)

Although the role of viruses remains unclear as risk factors in cancer, few evidences suggest that infection with high-risk HPV, particularly HPV-16 could have an impact on cancer occurrence (Herrero et al., 2003; Gillison and Lowy, 2004). The association of the virus was strongest for cancers of the oropharynx (D'Souza et al., 2007). Studies showing oral cancer as having a high risk in women with previous HPV-associated cancer, provided more evidences of link with HPV infection (Spitz et al., 1997; Hemminki et al., 1999). The presence or absence of HPV in patients with SCC of the head and neck is a new parameter for the prediction of long-term outcome of cancer of the oral cavity and of the oropharynx (Curado et al., 2009).
B. Wood and cement dusts

Another factor which has been investigated as related risk factors to develop head and neck cancer are environmental factor such as wood and cement dusts. A previous study has shown that these risk factors are related especially to OSCC (Blones, 2002).

2.4. Molecular basis and biology of oral cancer

An understanding of the molecular basis of oral carcinogenesis will be helpful for enhancing the clinical achievements, since it would provide crucial information in order to find out the best treatment for oral cancers once the successful diagnosis of the disease has been made. Knowledge of the basic molecular genetic events can help us to understand the disease process. Moreover, since oral cancer may result from various injuries and alterations to the genes, there could be different genetic pathways involved in its transformation from normal cell to malignant cells (Shetty, 2003). Hence knowing about the genetic pathways becomes a necessity.

Squamous cell carcinoma of the head and neck (HNSCC) is a heterogeneous disease with complex molecular abnormalities. It comes from a premalignant progenitor with abnormal proliferation of clonal cells, which are associated with genetic alterations and phenotypic progression to malignancy. These genetic alterations result in inactivation of some genes in the DNA repair pathways, which include the process that is supposed to rectify the damaged DNA in order to change the structure and function of the proteins which are important for cell mechanism. Genetic alterations can thus lead to inactivation of multiple tumor suppressor genes and activation of proto-oncogenes. Intramucosal migration
and clonal increase of transformed cells with formation of abnormal genetic fields appear to be responsible for local recurrence and development of secondary tumors in head and neck cancer (Ordonez et al., 2005).

2.5. Carcinogenesis

Carcinogenesis is a process by which normal cells are transformed to abnormal cells. Genetic events within the signaling transduction pathways that govern the normal cellular physiology are altered (Powell et al., 1993) in this complex multi-step process. The genetic basis of cancer is now well-established. Under normal conditions, these tightly controlled excitatory and inhibitory pathways regulate the oral keratinocyte biology. Basic cellular functions under these controls include cell division, differentiation, senescence and adhesion. These regulatory pathways are composed of extracellular ligands, which bind to the cell-surface receptors to control intracellular signals that are sent through secondary messengers for cells which are for better controlling of cell functions. These signals either directly alter cell function or stimulate the transcription of genes which affect on proteins (Bishop et al., 1991).

Cancer is actually the result of several changes occurring inside and outside of cellular pathways, which could occur at any level of these pathways. Mutations are necessary for genetic transformations. For transforming a normal cell into its malignant form it has been estimated that at least three to six somatic mutations are necessary (Powell et al., 1993). As the cell accumulates these alterations or mutations, it becomes functionally independent from the surrounding oral epithelium, which is made up of normal oral keratinocyte (Sidransky et al., 1995).
Normally the cellular functions are tightly controlled by these regulatory pathways. However, for tumor cells, they will proliferate abnormally, thus stimulate the neovascularization and continually grow by invading locally or metastasizing to distant sites (Owens et al., 1995). The histological progression of oral carcinogenesis is believed to reflect the accumulation of these changes (Field, 1995; Vokes et al., 1993).

Oncogenes, the gain-of-function in mutations of highly regulated normal cellular counterparts (proto-oncogene) are likely to be involved in the initiation and progression of oral neoplasia (Field, 1995). Cellular oncogenes were initially discovered by identifying the ability of tumor cell DNA to induce transformation of normal genes to abnormal forms (Pulciani et al., 1982). Mechanisms of activation of these cellular oncogenes include point mutations and DNA re-arrangements. Several of these cellular oncogenes are homolog of retroviral oncogenes (Field, 1995).

2.5.1. Oncogene

These are altered proto-oncogenes, which are the growth-promoting regulator genes (housekeeping genes). These genes promote cell growth and mitosis. Proto-oncogenes are conserved through evolution in nature (Shetty, 2003). They are components of metabolic processes i.e. cell proliferation and differentiation. These genes have important roles in cell functions because they code for the functional and the regulatory proteins, such as growth factor receptors, protein kinase, nuclear transcription factor and cell signaling transducer factor (Shetty, 2003).
2.5.2. Tumor suppressor gene

Tumor suppressor genes discourage cell growth unlike oncogenes. Accumulation of activated genes is an important factor for development of cancer, but these genes alone are not sufficient to result in oral cancer. So inactivation of negative regulatory tumor suppressor genes may also be required for oral cancer. Oncogenes show mutations at only one of the gene copies, while tumor suppressor genes are inactivated by point mutation, deletion and rearrangement in both gene copies. Thus they are difficult to identify because they are negative phenotype or no longer present within the cell. Only two genes, p53 is known for tumor suppressor activity in oral cancer. Deregulation of these genes may effect on cell cycle, chromosome stability, senescence, apoptosis and control of cell proliferation (Shetty, 2003).

2.6. Molecular epidemiology

Molecular epidemiology is a part of medical science that focuses on the contribution of genetic and environmental risk factors, identified at the molecular level, to the etiology, distribution and prevention of disease within families and inside populations. This field has emerged from the studies of molecular biology into concepts of epidemiologic research. Molecular epidemiology improves our understanding of pathogenesis of disease by identifying specific pathways, molecules and genes that has an influence on the risk of developing disease (Wacholder et al, 2004). In recent years, this field has increased the chances of detecting high-risk lesions within individual. The main goal of molecular epidemiology in oral cancer study is to determine whether prognosis of oral cancer patients are associated with the gene expression profiles. Molecular
epidemiology is also to determine the variation in gene expression of invasive OSCC, preneoplastic oral squamous cell lesions, and normal oral tissue (Chen et al., 2008)

2.6.1. Genetic susceptibility

In cancer etiology the role of genetics can be divided into two categories: the single (rare) genes and the more common susceptibility genes. The single (rare) genes are about the normal genes which, under endogenous and exogenous factors may transform into cancer genes, while more common susceptible genes are those genes which are susceptible to transformation. The high penetrance disease genes are uncommon (i.e. they have a low allele frequency, typically less or much less than 1%). If these genes are present, they cause a higher risk for a particular cancer (Sinha and Caporaso, 1999).

Early studies reported that any carcinogen requires metabolic activation in order to cause cancer and it was proposed that there could be genetic control of activation of metabolic pathways or elimination (Sinha and Caporaso, 1999). A broader appreciation of human carcinogenesis suggests categories of genes that go beyond metabolic activation/detoxification. These include genes that influence DNA repair, chromosome stability, the activity of oncogene or tumor suppressor genes, cell cycle control or signal transduction, hormonal or vitamin metabolism pathways, immune function and receptor or neurotransmitter action (Sinha and Caporaso, 1999).

An individual difference in susceptibility to chemical carcinogens is one of the most important factors in estimating the risks of human cancer (Sato et al., 1999). Many different types of host factors may be involved in the mechanism of difference in susceptibility,
including possible polymorphic changes of oncogene or carcinogens and predispositions due to impaired antibody production or cellular immunity. Since different individuals have different responses to the same carcinogens, it has been suggested that the genetic differences in controlling the metabolism of chemical carcinogens in different individuals are associated with different predispositions to cancers (Nakachi et al., 1991).

Both hereditary and environmental factors can cause head and neck carcinogenesis and their roles are difficult to explain individually. Several cancer prone syndromes are associated with an increased risk of head and neck cancer, including Lynch-II, Bloom syndrome, Fanconi anemia, ataxia telangiectasia and Li-fraumeni syndrome (Blones, 2002). Genetic susceptibility as a factor for head and neck cancer are more likely due to various degrees of DNA maintenance after exposure to tobacco carcinogens. Mutagen sensitivity tests which study polymorphism in DNA repair enzymes or in carcinogens metabolizing enzymes supports the role of heredity in HNSCC. Among the polymorphism tested, XRCC1, the Arg allele (Arg194Trp) and the Gln allele (Arg399Gln) are also linked to an increased risk of oral and pharyngeal cancers (Blones, 2002).
2.7. Genetic polymorphism

2.7.1. Definition of a Polymorphism

Polymorphism is a germline variation in the base sequence of the genetic code. Depending on the frequency of this variation, a heritable variation is named as polymorphism if it is present at an allele frequency higher than 1% in the general population. At lower frequencies it is named a germline mutation (Strachan and Read, 1996). Polymorphisms and germline mutations are present in the genetic code of every cell of an individual. There are also non-heritable alterations in the genetic side, acquired during the lifetime of an individual. These are named somatic mutations and are present within affected tissue only. Thus, somatic mutations can proliferate by clonal proliferation of the mutated cell. But it cannot be transmitted to the offspring of the affected individual because it is not germline mutation.

Mutations are associated with severe diseases, while polymorphisms are generally not so. Thus polymorphisms can remain and spread within a population and can transmit into the next generation easily, either because they have no major effect or because they were associated with a beneficial effect that may be counteracted by modern lifestyle (Ryke, 2006). Like a new environment needs some new qualifications, polymorphism can alter some old qualities and change it to be matched with the new environment. Mutations, at least those that bring a harmful influence before reproduction, tend to be selected against by evolution since cell has many repair pathways to delete mutations. Even if they do not disappear completely they may arise spontaneously in the new generation. Also some
harmful mutations persist in populations because they are associated with a selective advantage under certain environmental conditions (Richer and Chudley, 2005).

2.7.2. Single nucleotide polymorphism (SNP)

Genetic polymorphisms are common throughout the genome. The most common type of polymorphisms, single nucleotide polymorphisms (SNPs), can occur as frequently as 1 out of every 1000 base pairs in the human genome. There are probably more than 10 million SNPs in the human population (Syvanen, 2001). Polymorphisms can occur in both introns and exons of the genes. Polymorphisms especially within exons have an impact on the structure and function of the protein coded by the gene, particularly in those cases when the polymorphism leads to an amino acid exchange in the evolutionarily conserved domains. Amino acid exchanges can lead to different protein with different function or same protein with a silent function, or just changing the structure of the protein that could ultimately change the function as well (Takeshita et al., 1994).

For each polymorphism, one of the alleles is considered to be the main allele and this is usually named the wild-type allele. The wild-type is supposed to be the most common (normal) genetic variant, but since the frequencies varies this need not to be true for all populations. Alleles of SNPs that are close together tend to be inherited together. A set of such alleles in a region of chromosome is called a haplotype. Most chromosome regions have only few common haplotype (Jakobsson et al., 2008), which together accounts for most of the genetic variations from person to person in a population.
2.7.3. Importance of polymorphism

Life on earth and evolution are based on small genetic variations that occasionally arise and give the carrier an increased ability to survive and reproduce in specific environments in which the organisms exist. This environment may require the organism to have some new quality, which is necessary for interaction with an environment while another environment does not. These genetic variations may provide the organisms to have some new quality to survive in their new environment. The genetic variations are transferred to the offsprings and will spread during the following generations and increase the possibility for survival for the whole population in its current environment, and adapts this possibility for every future generation. Among these genetic variations are the polymorphisms. Many polymorphisms can spread in populations possibly because they have no or very little effect. However, some may also spread because they confer an advantage in certain situations (Ryke, 2006).

A polymorphism that leads to an amino acid exchange may change the nature of proteins. They are usually found within an active site of an enzyme, at a DNA binding site, a substrate binding site or in other areas of importance for the protein function and this could influence the activity of the encoded proteins. This is especially true if the new amino acids have a different three dimensional forms or electrical charge than the original amino acid, as this will change the structure or affinity of the protein and make it non-functional, or more or less efficient than the original proteins (Ali-Osman, et al 1997; Watson, et al 1998). If the proteins are involved in processes such as DNA repair, cell cycle control or metabolism of toxic substances; a change in function may be associated with a different
susceptibility to disease in a carrier of the variant allele (Ryke, 2006). Thus the susceptible individuals carrying the polymorphic variations will develop symptoms of intoxication at lower doses of exposures than individuals not carrying this allele (Tarlow et al., 1992; Wober and Hein, 1985; Takeshita et al., 1996). In fact, less efficient DNA repair may increase the risk of having a permanent damage, for example a mutated DNA base or micronuclei (Norppa et al., 2006).

Since mid 20th century, it has been known that people react differently to certain drugs, even if they have the same diagnosis. Later this was found to be associated with genetic polymorphisms in the genes that metabolized the active substances of the medicine (Fojo, 2001; Penas et al., 2006). Polymorphism may also help to identify the genetic traits of different diseases. For example, change in amino acid profile due to polymorphism can alter the function of a protein, which would be associated with a certain kind of disease. Therefore there is a high probability is there that this polymorphic gene could play an important role for that particular disease.

Another important aspect of studying polymorphisms is that they may help us to identify the importance of certain exposure in a population, which could help in planning health care. This type of information in future may allow for provision to individual some useful advices before disease diagnosis, or may offer ideas for personalized treatment as soon as the disease is diagnosed. In most cases the effect of a single polymorphism is weak. If an individual with a variant allele is susceptible to a certain exposure, they will be at an increased risk for diseases associated with the polymorphism. This is because the specific gene that has the polymorphism is related to that particular disease. Furthermore, if the
individual is not exposed to a substance which is associated with susceptibility, the polymorphisms will probably not have any effect (Takeshita et al., 1996).

The effect of polymorphism on health is often more complex. Therefore not only the environmental effects but also other factors like genes that may interact should also be taken into consideration. It is not so surprising that this complexity has resulted in conflicting results with many polymorphism studies (Pezzotti et al., 2009). However, even though the polymorphic effect on individuals is reasonable, it may be large at the population level. There are a number of studies that indicated a possible association between polymorphic variants of certain genes and disease risk (for example cancer risks) (Abdel-Rahman et al, 1998; Huang et al., 2006).

The frequency of different polymorphic variants varies with the ethnicity of a population in different parts of the world. Therefore the same exposures in different parts of world and also different ethnic groups within the same country may not give the same results. This is because of the fact that the area may be the same but the frequency of polymorphic variants varies among different ethnic groups. Knowledge of the frequency of polymorphisms in a population could therefore be important for understanding the impact of certain exposures on a population, which could ultimately be used for planning the health care in future.
2.7.4 Polymorphisms with impact on DNA damage and repair

2.7.4.1 DNA repair pathways

Approximately 10,000 spontaneous oxidative damages are known to occur in every human cell a day (Fortini et al., 2003). If a cell is not able to repair, it may lead to cell death or mutations and malignant transformation (cancer). There are a number of efficient DNA-repair mechanisms to protect the genomes in human. These systems can be divided into different pathways depending on the type of DNA lesion that was damaged. Generally, a polymorphism that decreases the ability and efficiency of a repair enzyme will increase the susceptibility for the type of damage that it is supposed to repair. Cells with defective DNA-repair mechanisms generally show increased sensitivity to genotoxic agents and increased mutation rate.

Individuals with inherited inabilities of DNA repair display increased sensitivity towards genotoxic exposure, increased levels of chromosomal aberration and mutation in somatic cells, which leads to cancer (Lehmann, 2003; Cleaver, 2005). There are many polymorphic DNA repair genes that are involved in DNA repair pathways including the base excision repair (BER), the nucleotide excision repair (NER), the homologous recombination repair (HR) and the miss-match repair (MMR) pathway.

Damage of single DNA base (example of damage by oxidative process is ionizing radiation) is repaired by the BER pathway which usually repair the damaged base to a normal base (Fortini et al., 2003). The BER process starts with the enzymatic removal of the damaged base by DNA glycosylases, which creates an abasic site that removes the
damaged purine or pyrimidine base and thus making an apurinic or apyrimidinic sites (AP). AP can also be produced spontaneously or after being under exposure to radiation or chemical. Some glycosylases (bifunctional glycosylases) have an associated apurinic/apyrimidinic lyase activity and they further catalyze the cleavage of the sugar-phosphate chain and excision of the abasic part of DNA, leaving a single nucleotide gap (Fortini et al., 2003). Other glycosylases (monofunctional-DNA-glycosylases) have no associated lyase activity. When such enzymes start repairing, the phosphodiester bond at the 5’ side of the intact apurinic/apyrimidinic site is incised by apurinic/apyrimidinic endonuclease (APEX1) (Fortini, et al., 2003). After this cleavage of the phosphodiester bond, the BER mechanism can proceed through two different sub-pathways: the short-patch BER and the long-patch BER. The first sub-pathway is characterized by the insertion of a single base at the lesion site that is without base. The long-patch BER involves the re-synthesis of a longer oligonucleotide spanning two to seven nucleotides in length. The gap is filled in and sealed by DNA-polymerases and DNA ligases (Fortini et al., 2003; Hung et al., 2005). The BER process can also be initiated by binding of the XRCC1-PARP (poly-(ADP-ribose)-polymerase) complex to a single strand breaks (Whitehouse et al., 2001). No human disorders have been related directly to inherited BER deficiencies until now. Earlier knockout mice that lack core BER proteins have resulted in death even at embryonic stages, and this confirms the importance of the role of BER process (Fortini et al., 2003).
2.8. X-Ray Cross Complementing group 1 protein (XRCC1)

XRCC1 is a DNA repair protein. This protein makes complexes with DNA ligase III. This protein is encoded by XRCC1 gene. XRCC1 gene is involved in the efficient repair of DNA single-strand breaks that are formed by exposure to ionizing radiations and alkylating agents day to day in every single cell. This protein interacts with DNA ligase III, polymerase beta and poly (ADP-ribose) polymerase (PARP) to participate in the base excision repair pathway, which is a pathway to repair the damaged base. This protein also plays a major role in DNA processing during meiogenesis and recombination in germ cells. Research studies suggest that a rare microsatellite polymorphism in this gene is associated with cancer patients of varying radio sensitivity (Hung et al., 2005).

2.8.1. XRCC1 as an important protein in DNA repair pathway

The repair of damaged DNA is essential for preventing any mutations to become fixed in the genome of a cell and increase after cell divisions or transmit to the next generation. In a population, the individuals with repair capacity may collect genetic alterations and this can most probably result in the increased risk of developing different kinds of cancer. Polymorphism can result in small structural alteration in the repair enzymes and therefore changes in the susceptibility to cancer (Benhamou and Sarasin, 2000). Functional polymorphism in DNA repair genes are thought to be responsible for inter-individual differences in repair capacities that exist within the population.

X-ray cross complementing group 1 protein (XRCC1) is important for genetic stability and for embryonic viability (Tebbs et al., 1999) and is involved in the repair of
DNA single-strands breaks and base damages from a variety of endogenous and exogenous oxidants. XRCC1 acts as a scaffold protein or as a coordinator in single strand break repair in BER. They interact with at least three other enzymes, poly-ADP-ribose polymerase (PARP), DNA ligase III and DNA polymerase-β (Shen et al., 1998; Hu et al., 2001). Three coding polymorphisms that could alter the XRCC1 function have been identified; at codon 194 (Arg to Trp) within a hydrophobic core in exon 6, at codon 280 (Arg to His) in exon 9 and at codon 399 (Arg to Gln) in exon 10 (Shen et al., 1998). Although all three of these polymorphisms lead to amino acid substitutions, they can affect the function and structure of the protein. However, there is no direct data on their functional consequences. Nevertheless, the common Arg 399 Gln variation, which occurs within the PARP binding domain is also located within the central BRCA1 (breast cancer susceptibility gene-1 product) at carboxyl-terminal protein interaction domain, which is required for efficient single-strand break repair (Sterpone et al., 2010).

XRCC1 codon 399 normally has Arg/Arg amino acid according to G/G (Guanine) bases in the genomic DNA. When restriction enzymes are used, they realize the region on this place and cut it into two bands (241 bp and 374 bp). But if it is in abnormal situation (Arg/Gln; Gln/Gln), it means that the second base of the genomic DNA is changed to A (Adenine). Thus restriction enzyme cannot realize the area and this area will stay undigested and thus the band will be in 615 bp because of no digestion. Other possibilities exist where some samples could be found in 3 bands after electrophoresis in 615, 374 and 241 bp. This means that some samples have equal G/G and G/A. Thus, restriction enzyme will cut those alleles that have G/A. Hence 3 bands are observed for this polymorphism type.
The AP endonuclease, APEX1, is the rate-limiting enzyme in the BER pathway (Ramana et al., 1998), where it assembles pol-β onto AP sites and allows pol-β and DNA ligase-III to enter DNA repair sites (Bennett et al., 1997). It is well-supplied in human cells and accounts for nearly all of the abasic site cleavage activity observed in cellular extracts. APEX1 expression is increased in a variety of cancers and high APEX1 expression has been associated with poor outcome of chemoradioterapy. There are a number of polymorphisms identified in the APEX1 gene. Three of them show reduced endonuclease activity (Hadi et al., 2000), but occurred at a rather low frequency. The APEX1 Asp 148 Glu polymorphism may not result in reduced endonuclease activity, but a reduced ability to communicate with other BER proteins that give rise to reduced BER efficiency has been suggested (Hadi et al., 2000). The variant 148 Glu allele has also been reported to be associated with a higher sensitivity to ionizing radiations (Hu et al., 2001).

2.8.2. The role of XRCC1 in Cancer

Several functional polymorphisms (Arg194Trp, Arg280His and Arg399Gln) in the X-ray repair cross-complementing group 1 (XRCC1) have been identified to be associated with cancer risk. Human cancer can be started by DNA damages that can happen either by UV ionizing radiation and environmental chemical agents.

To protect the genome, different kind of DNA repair systems have been developed. Among many DNA repair mechanisms in mammals, base excision repair is the primary guardian against damages that results from cellular metabolism, including reactive oxygen species, methylation, deamination, and hydroxylation. Therefore, base excision repair is an important event in the cells and it is also required for preventing mutagenesis (Zhibin et al., 2001).
X-ray repair cross complementing group 1 (XRCC1), is one of the 20 genes that participate in base excision repair pathway. They produce a scaffolding protein that is a part of repairing the single-strand breaks, which are the most common lesions in cellular DNA (Caldecott et al., 1996). Both biological and biochemical evidence shows a direct role for XRCC1 in base excision repair because it interacts with a complex of DNA repair proteins.

The Arg399Gln polymorphism is located in the region of the BRCT-I interaction domain of XRCC1 within a poly (ADP-ribose) polymerase binding region, this polymorphism has been widely investigated with its association on cancer risk. The presence of the variant Gln399 allele has been shown to be associated with a large decrease of DNA repair capacity (Leng et al., 2005). A large number of molecular epidemiologic studies have investigated the role of the Arg399Gln polymorphism on cancer risk.
CHAPTER 3

METHODOLOGY

3.1. Study design

The present research was a case-control study conducted among three different ethnic groups in Malaysia. Cases are patients with oral cancer and those without cancer were taken as control groups.

3.2. Sample size estimation

Using the Epi Info Version 3.3.2, the sample size was determined for all objectives. Estimation based on the objective to investigate the association between XRCC1 Arg399Gln genotypes and oral cancer risk yielded affordable sample size. The estimation took into account the requirement for significance level of 0.05 and having at least 90% power of study. The ratio of control to cases was 1:1 and the odds ratio (OR) of exposure among cases for detection was 0.45. The prevalence of XRCC1 polymorphism among the controls was 85% (Sturgis et al., 1999). Finally, the estimated sample sizes were 203 subjects for each case and control group.

3.3 Population and samples

3.3.1 Reference population

The reference population to which the results of this study would be inferred to was all the oral cancer patients in Malaysia.
3.3.2 Source population

The source population for cases and controls were selected from the database of Oral Cancer Research and Coordinating Centre (OCRCC), University of Malaya. This database at OCRCC is the Malaysian Oral Cancer Database and Tumor Bank System (MOCDTBS). The system comprises of detail information of related parameters which were drawn out from the patients who have been diagnosed at selected centers namely the Dental Faculty of University Malaya (UM), Universiti Sains Malaysia (USM), Universiti Kebangsaan Malaysia (UKM) and the Ministry of Health Malaysia dental specialist clinics located at the general hospitals of Kuala Lumpur, Selangor, Perak, Kelantan, Sabah and Sarawak. MOCDTBS data was collected in a standardized manner pertaining to socio-demographic background, risk habits (smoking, drinking alcohol and chewing betel-quid), diagnosis, clinical staging, histological grading and follow-up information for future auditing to assess the disease outcomes and behavior.

3.3.3 Sampling frame

All patients that fulfilled both the inclusion and exclusion criteria have formed the sampling frame for the study.

3.3.4 Inclusion and Exclusion criteria

Patients who have been included as cases were:

- Patients who were diagnosed pathologically with squamous cell carcinoma of the oral cavity (OSCC) from the nine selected centers.
• Patients who have not been treated before, since treatment drugs could interact with genetic metabolism.

• Patients with either genomic DNA in the nucleic acid bank (at OCRCC-UM and CARIF- Cancer Research Initiatives Foundation) or blood samples.

Patients who have been included as controls were:

• Patients who did not have oral cancer, potentially malignant lesions or other kind of cancers.

• Patients with no prior history of cancer.

• Patients with no family history of cancer.

• Patients with either genomic DNA in the nucleic acid bank (OCRCC-UM and CARIF) or blood samples.

Patients who were excluded from being cases were:

• Non-Malaysian citizens

• Patients with recurrence

Patients who were excluded from being controls were:

• Patients with history of cancer.

For both case and control groups, patients and non-cancer individuals without genomic DNA in the nucleic acid bank (OCRCC-UM and CARIF) were also excluded.
3.3.5 Samples

Informed and written consent was obtained from all subjects participating in this study. Informed consent was obtained from the umbrella project titled “Oral Cancer in Malaysia, Risk Factors, Prognostic Markers, Genetic Expression and Impact on Quality of Life”, IRPA RMK 8 Project No: 06-02-03-0174 PR 0054/05-05. The ethical approval for the umbrella project was obtained from the Medical Ethics Committee, Faculty of Dentistry, University of Malaya Medical Ethics code No: DF OP0306/0018/(L) and was endorsed by the Ministry of Health, Malaysia. The study comprised of 209 cases and control group comprised of 212 patients selected from the MOCDTBS database. The overall methodology of the present study is summarized as flow chart in Figure 4.1:
Reference Population:
Oral cancer patients in Malaysia

Source population: All patients with records in the OCRCC-UM database

Source population of cases
Inclusion and Exclusion criteria for case and control
Sampling frame of cases: all patients that fulfilled both inclusion and exclusion criteria as cases
Sample: 209 cases with informed and written consent

Inclusion and Exclusion criteria for case and control
Sampling frame of control: All patients that fulfilled both inclusion and exclusion criteria as controls
Sample: 212 controls with informed and written consent

All patients were included as study subjects

Data collection:
Assessment on exposure to the risk factors of interest

Data entry and analysis using SPSS software version 18.0

Fig 3.1: Flowchart showing overall design of the study.
3.4 Variables in the study

The outcome or the dependent variable was occurrence of oral cancer. Risk factors of interest were grouped into genomic polymorphism and ethnic group.

Fig. 3.2: ¹ as dependent variable, ² as independent variables.

### Genetic polymorphism:

- Gln/Gln and Arg/Gln type of polymorphism

### Ethnicity

3.5 Data collection

Socio-demographic data namely habits, age, gender and ethnic of cases and controls were collected from the MOCDTBS.
3.6 Measurement Tool – XRCC1 Arg399Gln genotypes determination

Determination of XRCC1 Arg399Gln genotypes involved the isolation of genomic DNA followed by genotyping to confirm whether genes are present or absent, and finally to categorize XRCC1 Arg399Gln into different genotypes.

3.6.1 Isolation of genomic DNA (Extraction)

The entire genomic DNA used in this research was obtained from the nucleic acid bank from OCRCC-UM and CARIF (Cancer Research Initiatives Foundation). The genomic DNA was extracted using QIAamp Blood Mini Kit (Qiagen, Inc, Chartworth, CA) following the manufacturer’s instructions with slight modifications during the final elution step, where 200 µl of Buffer AE was added to the QIAamp Spin Column to increase the final DNA concentration in the elute. For each of the genomic DNA obtained, both the $A_{260/280}$ ratio and the quantity of DNA (ng/µl) were recorded using Nanophotometer which is a UV/Vis nano-volume spectrophotometer for quantitative and qualitative analysis of nucleic acid samples in a submicroliter volume range. After determining the concentrations of the samples, if DNA were not enough for genotyping, amplification of the DNAs was done. To prevent contamination and cross-contamination between the samples during PCR, extreme care was taken by preparing the samples for amplifying separately during DNA extraction.
3.6.2 Genotyping of XRCC1 Arg399Gln

Genotyping is done by using the PCR technique. A single or few copies of DNA was amplified into millions or more copies of a particular DNA sequence.

Table 3.1: Primer sequences for XRCC1 Codon 399

The primer sequence used for genotyping is as below:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Primer sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC1 Codon 399</td>
<td>Forward: 5´-TTG TGC TTT CTC TGT GTC CA-3´</td>
</tr>
<tr>
<td></td>
<td>Reverse: 5´-TCC TCC AGC CTT TTC TGA TA-3´</td>
</tr>
</tbody>
</table>

Details of the genotyping technique is as in Appendix 1.

3.6.3 Categorizing of XRCC1 Arg399Gln using the PCR-RFLP

The gene of interest was amplified in order for it to be large enough to be visualized on an Agarose or acrylamide gel, stained with ethidium bromide. Restriction enzymes that cleave the DNA specifically for different alleles were used to digest the DNA, and the allele of each sample was observed as a specific band pattern on the gel.
(i) Restriction Fragment Length Polymorphism (RFLP) Analysis

Restriction enzyme (RE) digest named Msp1 was used to distinguish the genotypes of XRCC1. Samples which were amplified in PCR reaction were used for restriction enzyme (Msp1) digestion. The detailed method of digesting the PCR Product with restriction enzyme and the preparation of RE Mix is as in Appendix 2.

(ii) Agarose gel and Electrophoresis

XRCC1 genes in the PCR products were separated into the normal/wild type (Arg/Arg genotype) variant and two polymorphism variants namely the Arg/Gln genotype and the Gln/Gln genotype. The agarose gel was used to isolate the PCR and PCR-RFLP products. Agarose gel electrophoresis visualized under the UV transmission will show bands indicating the corresponding base pair for the respective genotype of interest. In the second stage of electrophoresis, after using Restriction Enzyme and incubating overnight, 3 types of XRCC1 genotypes were identified. Table 3.2 showed the criteria in determining the genotypes of the XRCC1 Arg399Gln. Preparation of Agarose gel is shown in Appendix 3.
Table 3.2: Criteria for determination of the genotypes of XRCC1 Arg399Gln.

<table>
<thead>
<tr>
<th>Normal/wild type</th>
<th>Polymorphic types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arg/Arg Genotype Homozygote</td>
<td>Arg/Gln Genotype Heterozygote (Polymorphism)</td>
</tr>
<tr>
<td>241 bp</td>
<td>241 bp</td>
</tr>
<tr>
<td>374 bp</td>
<td>374 bp</td>
</tr>
<tr>
<td></td>
<td>615 bp</td>
</tr>
</tbody>
</table>

3.7. Statistical Analysis

Data entry and data analysis was done using Statistical Programme for Social Science (SPSS Version 18.0, Chicago, USA). Data was first checked and cleaned. Descriptive statistics such as mean, standard deviations or median and inter quartile range (IQR) were used to describe all continuous variables. Categorical variables were calculated as frequency and percentages. Analysis was done according to the specific objectives of the study. To achieve all the objectives, chi square tests were performed and odds ratio were computed. The level of significance was set at 0.05.
CHAPTER 4
RESULTS

4.1. Socio-demographic profile of cases and controls

This case-control study involved 209 cases of oral squamous cell carcinoma and 212 controls with no history of any kind of cancers or any family history of cancer. All the analysis was done based on these unmatched case-control study subjects. All selected socio demographic profiles of cases and controls were summarized in Table 4.1.

Mean age for cases was 61.34 ± 14.01 years and the mean age for controls was 45.56 ± 12.03 years. When gender distribution was considered, in the cases where 209 patients were studied, the percentage of female was higher (62.7%) than males (37.3%). For control group, the percentage of female was slightly higher (54.2%) than males (45.8%). Hence for both cases and control groups, there were more female than male. However, when gender distribution was compared, there is no statistically significant difference between the 2 groups ($p=0.079$). When different ethnic groups in Malaysia such as Malays, Chinese and Indians were considered, highly significant result was detected between the cases and control groups ($p=0.001$). Among the ethnic groups, Indians and Chinese were more among the cases, while Malays dominated the control group.

Similar pattern of distribution to the ethnic groups, were also detected in the other three significant variables such as smoking status, alcohol consumption and betel quid chewing status. Statistically significant differences in habit patterns between the case and
control groups ($p=0.001$ in smoking and alcohol drinking status; $p<0.001$ in betel quid chewing status) were observed.
Table 4.1: Socio-demographic profile of study subjects.

<table>
<thead>
<tr>
<th>Socio-demographic profile</th>
<th>Oral cancer status</th>
<th>$p$-value$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>78(37.3)</td>
<td>97(45.8)</td>
</tr>
<tr>
<td>Female</td>
<td>131(62.7)</td>
<td>115(54.2)</td>
</tr>
<tr>
<td>Ethnic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malay</td>
<td>91(43.5)</td>
<td>157(74.1)</td>
</tr>
<tr>
<td>Chinese</td>
<td>26(12.4)</td>
<td>14(6.6)</td>
</tr>
<tr>
<td>Indian</td>
<td>92(44.0)</td>
<td>41(19.3)</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>117(56.0)</td>
<td>154(72.6)</td>
</tr>
<tr>
<td>Currently smoking</td>
<td>55(26.3)</td>
<td>41(19.3)</td>
</tr>
<tr>
<td>Stopped smoking</td>
<td>37(17.7)</td>
<td>17(8.0)</td>
</tr>
<tr>
<td>Alcohol drinking status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never dranked</td>
<td>121(57.9)</td>
<td>180(84.9)</td>
</tr>
<tr>
<td>Currently drinking</td>
<td>54(25.8)</td>
<td>20(9.4)</td>
</tr>
<tr>
<td>Stopped drinking</td>
<td>34(16.3)</td>
<td>12(5.7)</td>
</tr>
<tr>
<td>Betel quid chewing status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never chewed</td>
<td>85(40.7)</td>
<td>161(75.9)</td>
</tr>
<tr>
<td>Currently chewing</td>
<td>89(42.6)</td>
<td>38(17.9)</td>
</tr>
<tr>
<td>Stopped chewing</td>
<td>35(16.7)</td>
<td>13(6.1)</td>
</tr>
</tbody>
</table>

$^a$ Chi square test was used. Level of significance was set at 0.05.
4.2 Distribution of XRCC1 Arg399Gln genotypes among cases and controls

Figure 4.1 illustrates the banding patterns of undigested PCR products of XRCC1 codon 399 genotypes and Figure 4.2 illustrates the banding patterns of the MSP1-digested PCR amplifications product of XRCC1 codon 399 to categorise the XRCC1 Arg399Gln into different genotypes.

Fig. 4.1: Banding patterns of undigested PCR products of XRCC1 codon 399 genotypes. Lane Ladder: 100bp DNA Ladder (Promega, USA): Lane 1, 2, 3 are samples in 615 bp; Lane –ve is the negative control.
Fig. 4.2: Banding patterns of the MSP1-digested PCR amplifications product of XRCC1 codon 399. Lane Ladder: 100bp DNA Ladder (Promega, USA): Lane 7: sample in 615 bp means Gln/Gln type of polymorphism; Lane 8, 9, 12: samples are setting in 241 and 374 bp, it means Wildtype or normal (Arg/Arg); Lane 10, 11, 13, 14, 15, 16: samples are setting in 241, 374 and 615 bp, which means Arg/Gln type of polymorphism; Lane PCR: PCR product as positive control.

When two types of polymorphisms were combined together the distribution of XRCC1 polymorphism (Arg/Gln; Gln/Gln) was 65.1% in the cases and 58.5% in the control group (Table 4.2). Even after the separation of two types of polymorphisms, the distribution of Arg/Gln was still higher among the cases (48.8%) as compared to control groups (41%). However, distribution of Gln/Gln was similar between the control (17.5%) and cases (16.3%).
Table 4.2 Distribution of XRCC1 Arg399Gln genotypes among cases and controls.

<table>
<thead>
<tr>
<th>Genotyping status</th>
<th>Oral Cancer Status</th>
<th>Frequency (%)</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td></td>
<td>Frequency (%)</td>
<td>Frequency (%)</td>
<td></td>
</tr>
<tr>
<td>XRCC1 genotypes (combined)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Arg/Arg)</td>
<td>73 (34.9)</td>
<td>88 (41.5)</td>
<td></td>
</tr>
<tr>
<td>Polymorphism (Arg/Gln;Gln/Gln)</td>
<td>136 (65.1)</td>
<td>124 (58.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>209 (100.0)</td>
<td>212 (100.0)</td>
<td></td>
</tr>
<tr>
<td>XRCC1 genotypes (individual)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Arg/Arg)</td>
<td>73 (34.9)</td>
<td>88 (41.5)</td>
<td></td>
</tr>
<tr>
<td>Polymorphism (Arg/Gln)</td>
<td>102 (48.8)</td>
<td>87 (41.0)</td>
<td></td>
</tr>
<tr>
<td>Polymorphism(Gln/Gln)</td>
<td>34 (16.3)</td>
<td>37 (17.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>209 (100.0)</td>
<td>212 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

4.3 Associations between XRCC1 Arg399Gln genotypes and Oral cancer risk

Table 4.3 shows the association between genotyping status and oral cancer risk. Chi square test revealed that either individually or combined, there was no significant association between XRCC1 Arg399Gln genotypes and oral cancer risk ($p = 0.165$, 0.260 respectively). Thus indicating that having the polymorphism does not mean a person has higher risk of getting oral cancer.
<table>
<thead>
<tr>
<th>Genotyping status</th>
<th>Case</th>
<th>Control</th>
<th>OR</th>
<th>95%CI</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Freq (%)</td>
<td>Freq(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XRCC1 genotypes (combined)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Arg/Arg)</td>
<td>73 (45.3)</td>
<td>88 (54.7)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphism (Arg/Gln; Gln/Gln)</td>
<td>136 (52.3)</td>
<td>124 (47.7)</td>
<td>1.322</td>
<td>(0.891 , 1.961)</td>
<td>0.165</td>
</tr>
<tr>
<td>Total</td>
<td>209 (49.6)</td>
<td>212 (50.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XRCC1 genotypes (individual)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Arg/Arg)</td>
<td>73 (45.3)</td>
<td>88 (54.7)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymorphism (Arg/Gln)</td>
<td>102 (54.0)</td>
<td>87 (46.0)</td>
<td>1.108</td>
<td>(0.633 , 1.938)</td>
<td>0.260</td>
</tr>
<tr>
<td>Polymorphism (Gln/Gln)</td>
<td>34 (47.9)</td>
<td>37 (52.1)</td>
<td>0.784</td>
<td>(0.454 , 1.354)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>209 (49.6)</td>
<td>212 (50.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Chi square test was used. Level of significance was set at 0.05
4.4 Distribution of XRCC1 Arg399Gln genotypes between three ethnic groups in Malaysia

Table 4.4 showed that there was no significant difference observed in the distribution of XRCC1 Arg399Gln genotypes either as individual ($p = 0.617$) or when combined polymorphism ($p = 0.641$) between three different ethnic groups.

Table 4.4 Comparison in distribution of XRCC1 Arg399Gln genotypes between three different ethnic groups in Malaysia

<table>
<thead>
<tr>
<th>Genotyping status</th>
<th>Race</th>
<th></th>
<th></th>
<th>$p$-value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Malay (Freq%)</td>
<td>Chinese (Freq%)</td>
<td>Indians (Freq%)</td>
</tr>
<tr>
<td>XRCC1 genotypes (combined)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Arg/Arg)</td>
<td>99 (39.9)</td>
<td>13 (32.5)</td>
<td>49 (36.8)</td>
<td>0.617</td>
</tr>
<tr>
<td>Polymorphism (Arg/Gln ; Gln/Gln)</td>
<td>149 (60.1)</td>
<td>27 (67.5)</td>
<td>84 (63.2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>248 (100)</td>
<td>40 (100)</td>
<td>133 (100)</td>
<td></td>
</tr>
<tr>
<td>XRCC1 genotypes (individual)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (Arg/Arg)</td>
<td>99 (39.9)</td>
<td>13 (32.5)</td>
<td>49 (36.8)</td>
<td>0.641</td>
</tr>
<tr>
<td>Polymorphism (Arg/Gln)</td>
<td>110 (44.4)</td>
<td>17 (42.5)</td>
<td>62 (46.6)</td>
<td></td>
</tr>
<tr>
<td>Polymorphism (Gln/Gln)</td>
<td>39 (15.7)</td>
<td>10 (25.0)</td>
<td>22 (16.6)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>248 (100)</td>
<td>40 (100)</td>
<td>133 (100)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Chi square test was used. Level of significance was set at 0.05
5.1 Distribution of Socio-demographic profile

For gender variants, there was no significant difference between males and females among the cases and controls ($p = 0.079$). But a previous study done by Suba (2007) have pointed out that oral cancers have fairly high male to female ratio in most populations. The report suggested that the lower incidence of oral cancer among women than man is because of the involvement of certain endocrine factors during its development (Suba, 2007). According to Parkin et al.,(2005), incidence of oral cancer among females is higher in Southern Asia (8.3 per 100,000). This pattern is because of prevalence of specific risk factors such as betel quid chewing in Southcentral Asia and Melanesia. Moreover, significant differences among gender may be different in different ethnic groups, because of various factors like different geographical areas, genetic susceptibilities and different lifestyles. Another important reason behind this variation could be dependent upon the gender that indulged most in risk habits already known to be related to oral cancer such as smoking, alcohol drinking and betel quid chewing

In the present study there is a statistically significant difference in the distribution of cases and controls in the Malays, Chinese and Indians ($p< 0.001$). Among the cases, the Indians were the predominant group (44.0%), which was followed by the Malays (43.5%) and finally the Chinese (12.4%). This pattern of ethnic distribution as observed in the present study among cases was also observed in earlier studies on mouth and tongue
cancers where a similar trend was observed with the highest incidence being in the Indians (47.9%), followed by Malays (26.2%) and Chinese (26.0%) (Lim and Yahaya, 2004).

High rates of oral cancer have been reported from South Asian countries like Nepal, Indian and Sri Lanka (Moore et al., 2009). A previous study by Ramanathan and Lakshimi (1976) had reported the prevalence of oral cancer is low among the Malays and Chinese but high (60%) among the Indians. Their results had been confirmed by other later studies that Indians are more susceptible to have oral cancers than other ethnic groups in Malaysia (Lim and Yahaya, 2004). This trend had been attributed to the fact that more Indians indulged in risk habits such as betel quid/areca nut, and/or usage of other tobacco products (Ramanathan and Lakshimi, 1976). Even though it has been reported that the incidence of oral cancer in Malays is low compared to the Indians, a report by Abdul-Razak et al (2009), showed an increase in the number of Malay patients diagnosed with oral cancer over a twenty-year period from 1986 to 2005 in Hospital University Sains Malaysia, which was mainly because of the increased habit of smoking among the Malays.
5.2 Distribution of XRCC1 Arg399Gln genotypes

The results from this study revealed that those patients who carried the Arg/Gln type of XRCC1, has a higher distribution, therefore they could be possibly more susceptible to oral cancer as compared to others. On the other hand the distribution of the second polymorphic type of XRCC1 (Gln/Gln) showed lower distribution among cases (16.3%) as compared to controls, which have higher distribution of this polymorphic type (17.5%). However, both these distributions (16.3% and 17.5%) were almost similar (Table 4.2.). These genes may have been susceptible either by their own way or due to the effect of certain habits like smoking. Since smoking habit is high in Malaysia, this could interact with this polymorphic type (Arg/Gln) and may ultimately lead to higher risk of oral cancer (Ito et al., 2004).

From our study it was also observed that distribution of the polymorphic type Gln/Gln, have less influence on oral cancer. When we separated two types of polymorphisms and compared the frequencies, distribution of Gln/Gln was lower than distribution of Arg/Gln among both cases and controls. Therefore may indicate that Gln/Gln polymorphic type has no influence on oral cancer in our study, but according to the distribution of Arg/Gln, this type of polymorphism might have an influence on oral cancer. Huang et al. (2006) have studied the distribution of different genetic polymorphisms among selected cases and controls. One of the genes they have investigated was XRCC1. In their results they had found that XRCC1 genotype such as Arg/Arg, Arg/Gln and Gln/Gln among cases showed distribution of approximately 47.9%, 39.4% and 7.2%, respectively. But among control group, the genotype; Arg/Arg, Arg/Gln and Gln/Gln showed
distribution of 42.6%, 42.6% and 10.2%, respectively. Huang et al. (2006) showed that the
distribution of wild type (Arg/Arg) was higher among cases, but the distributions of both
types of polymorphisms (Arg/Gln, Gln/Gln) were higher among controls. In their study,
Huang et al. (2006) have showed that having polymorphic genotypes of XRCC1 genes do
not lead to a harmful effect on susceptibility for oral cancer risk among American
population. But in our study having polymorphic type (Arg/Gln) may lead to a harmful
effect in view of the fact that the distribution is higher in cases than in controls.

The differences between distribution of wildtype (Arg/Arg) and polymorphic type
(Arg/Gln) among cases and controls in our study as compared to Huang et al. (2006) was
because of the variation in the susceptibility at different environments for different ethnic
groups as Huang et al.’s study was conducted among the American population while this
current study was conducted among Malaysian population. Another reason could be
because of the different habits among patients. Huang et al.’s (2006) research had shown an
opposite observation as compared to our study, except for distribution of polymorphic type
(Gln/Gln), which was also high among controls in our study. Hence, it could be concluded
that the polymorphic type Gln/Gln has least harmful effect on patients of Malaysian and
American population. In another study, Sturgis et al (1999) have compared the distribution
of genotypes and reported a distribution of 46.3%, 37.9% and 15.8% for Arg/Arg, Arg/Gln
and Gln/Gln among cases, respectively. For the control group, the distribution was 42.7%,
46.5% and 10.8% for Arg/Arg, Arg/Gln and Gln/Gln, respectively which was opposite to
the results of the current study. Sturgis et al’s study also highlighted that different ethnicity
and environment may have effect on susceptibility of genes.
As it is mentioned above, in the current study, considering the higher distribution of polymorphic type Arg/Gln among cases as in table 4.2, it showed a trend towards having an impact on oral cancer. In addition, the distribution of Arg/Gln was higher among the cases. This finding may indicate that this polymorphic type of XRCC1 might have an influence on oral cancer. The distribution of another polymorphic type of XRCC1 namely, Gln/Gln, however, showed a trend towards having no influence on oral cancer risk. Gln/Gln also has higher distribution among controls as compared to its distribution among cases, thus indicating that Gln/Gln does not have a harmful impact on oral cancer.

5.3 Association of XRCC1 Arg399Gln genotypes and oral cancer risk

In the present study the genotyping status with oral cancer groups was not statistically significant for both when the 2 types of polymorphism were (p= 0.165) and when considered separately (p = 0.260) (Table 4.3). The results from this study revealed that the Gln/Gln polymorphism has decreased risk to oral cancer where the odds ratio was 0.784 (95% CI: 0.454, 1.354) as compared to Arg/Gln genotypes, which showed an increased risk to oral cancer status where the odds ratio was 1.108, (95% CI: 0.633, 1.938). When both types of polymorphisms were combined, the odds ratio was 1.322 (95% CI: 0.891, 1.961) indicating an increased risk to the oral cancer as compared to the Arg/Gln genotypes. However, the results were not statistically significant.

In contrast, previous studies had shown a risk to oral cancer of the polymorphic type Gln/Gln where they had shown that the odds ratio were 1.34 (95% CI: 0.80, 2.24) (Sturgis et al, 1999), 1.28 (95% CI: 1.05, 1.57) (Azad et al, 2011) and 1.32(95% CI: 0.57, 2.08) (Varzim et al, 2003). However, Olshan et. al. (2002) reported a decreased risk for oral
Yen et al. (2008), had found there is an association between XRCC1, XRCC2, XRCC3, XRCC4 polymorphisms and oral cancer risk among Taiwanese population. Ramachandran et. al. (2006) had shown the presence of the polymorphic variant of XRCC1 codon 399 and XPD was associated with increased risk of oral cancer compared to the wild genotype among Indian Population.

Among some other studies that have investigated on the association of XRCC1 Arg399Gln and other cancers, some have reported that there was an increased risk of having lung cancer by carrying the XRCC1 Arg399Gln (Lunn et al., 1999; Divine et al., 2001). In an investigation done by Sreeja et al. (2008), association between polymorphic genotype of XRCC1 Arg399Gln have reported an incidence of elevated risk of lung adenocarcinoma (OR: 2.5, (95% CI: 1.1-5.8)). Their findings suggest that the XRCC1 mechanism and the role of this gene on DNA repair have variations at different locations or organs of the human body. For example Divine et al., (2001) reported that the polymorphic type Gln/Gln have no harmful effect on oral cancer but have an effect on risk of having lung cancer. This explains that the polymorphic type Gln/Gln has a least effect on DNA repairing in the lungs. Therefore patients who seem to have polymorphic Gln/Gln polymorphisms could possibly have less ability to repair their DNA once it is damaged. Another possible reason for those who have less ability to repair the DNA damages in lung area could be because of the effect of certain habits such as smoking, which can damage DNA in lung area more than other parts of body.

It can also have a possible ability to change the wild type to the polymorphic types, which have less sufficient effect on repairing damaged DNA to prevent cancer.
development. The mechanism of XRCC1 in DNA repair could be different in different parts of the body. One of the reasons behind this difference is that some other genes could interact directly on mechanisms of XRCC1, which may be active or inactive in different parts of the body. Moreover, different exposures such as smoking, radiation, etc. can have different influence on DNA repair mechanisms in different part of body. Some exposures can lead to the DNA repair mechanisms being more effective while some other exposures can make this mechanism weak and insufficient.

In this study, however the association between XRCC1 polymorphism and oral cancer risk was done for all ethnicities as overall. Even though, a significant difference in distribution of Malays, Chinese and Indians was noted between cases and controls, the association between XRCC1 polymorphism and oral cancer risk stratified for each ethnic was not possible due to limited sample number. Thus, the interpretation of no significant association between XRCC1 polymorphism and oral cancer risk found in this study, has to take into account this limitation.

5.4 Distribution of XRCC1 Arg399Gln genotypes and three ethnic groups in Malaysia

The association between ethnic groups and genotyping status was not statistically significant whether the XRCC1 codon399 polymorphisms were considered separately (p=0.617) or in combination (p=0.641) (Table 4.4). However, odds ratio in the present study suggested an increased risk for XRCC1 Arg399Gln polymorphism. The current study was the first effort to study widely on the association of XRCC1 in the Malaysian population. Similar attempt was done by Olshan et.al. (2002), on white and black populations in USA. They showed a decreased risk of having oral cancer for the
Arg399Gln polymorphism (OR=0.6; CI=0.4–1.1) for America population. Furthermore, they reported that the odds ratio for the Gln/Gln genotype among whites (OR=0.1; CI=0.04–0.6) and blacks (OR=0.01; CI=0.0004–0.3) showed a decreased risk of having oral cancer substantially. They also investigated the interaction between the Arg194Trp and Arg399Gln polymorphisms on the usage of tobacco, where they recommended that additional epidemiologic and functional studies were required to resolve the importance of XRCC1 polymorphisms in SCCHN.

Ramachandran et al (2006), in their attempt to study the distribution of XRCC1 Arg399Gln genotypes on Indian population have found that the presence of the polymorphic variant of XRCC1 at codon 194 and 399 was associated with increased risk of oral cancers as compared to the wild genotype. In another study, Tae et al (2000) have reported that association of the XRCC1 polymorphism with SCCHN could be considered as markers for genetic susceptibility among the Asian population, especially Chinese and Indians. Another study done among Chinese population on the association between DNA repair genes XRCC1 Arg194Trp with oral cancer risk suggested that there were no evidence for an effect on the risk of oral cancer (Zhibin et al., 2005). However, they have found some evidence for effect with interaction factors such as ethnicity and smoking status.

One recent study by Geng et al (2009) on the prevalence of Arg399Gln and prostate cancer was conducted, where they selected their samples from United States, Japan and China. In their study the prevalence of Arg/Arg (wildtype) was 50%, 46% and 73% among Asian, white and African descent, respectively which revealed that the wild type was
highest among the Africans and lowest among the whites. The prevalence of Arg/Gln genotypes were 38%, 42% and 24% among Asian, white and African descent, respectively. This showed that this polymorphic type was higher among the whites and lowest among the African descents. The prevalence of Gln/Gln were 12%, 12% and 3% among Asians, whites and Africans, respectively which states that this Gln/Gln polymorphic type had lowest incidence among the Africans. They suggested that XRCC1 Arg399Gln polymorphism was unlikely to be a major risk factor for prostate cancer among Asian population. However, genotype Gln/Gln allele has shown 43% more likely to have prostate cancer among Asians than those with Arg/Gln and Arg/Arg alleles (Geng et al., 2009).

In another study by Huang et al. (2009), the association between Arg399Gln polymorphism and breast cancer was investigated, which showed that Arg399Gln polymorphism increased the chances of breast cancer risk in Asians (OR: 1.26, 95% CI: 0.96-1.22) and Africans (OR:1.80, 95% CI: 0.97-3.32). They also found that the chances of breast cancer risk in Caucasians were comparatively lesser than the other groups, but still there was a slight increasing of susceptibility in this population (OR: 1.08, 95% CI: 0.95-1.22). They suggested that the increased risk of breast cancer risk among the Asians and Africans were related to habits such as smoking and chewing areca nut. These kinds of habits have already been shown to have critical influence over the interaction between polymorphic types of XRCC1 and different types of cancers. They have also suggested that the usage of tobacco or chewing betel quid can have interaction on Arg399Gln, which could change the polymorphic type (Gln/Gln) to another polymorphic type (Arg/Gln), that apparently have increased risk for cancer. This could increase the susceptibility of XRCC1 to having cancer among Asians than other populations.
Roberts et al. (2011) have investigated the association of single nucleotide in DNA repair genes and breast cancer through meta-analysis. They showed that there were no association between XRCC1 rs25487 (Commonly known as Arg399Gln) and breast cancer risks in Caucasians. Similar to this increased risk associated with this polymorphism was also reported among Iranian and Portuguese population (Roberts et al., 2011). In general from all these studies, it could be concluded that different environmental effects could influence the DNA repairing mechanisms in different ways among various ethnic groups in different countries. In conclusion, factors like; life styles, nutrition, habits, the effect of environment and many other elements can lead to different effect on DNA repairing mechanisms of XRCC1 among different ethnic groups.

5.5 Limitation of the study

The limitation of this study was the ethnic composition of the sample, which did not reflect the true population of the ethnic groups in Malaysia. The sample size was selected according to other similar studies reported in the literature, which for most countries represent one ethnic group as compared to a country like Malaysia with many ethnic groups. The number that we had taken in this study could have been more than enough as a suitable sample size for a country with only one ethnic group or in a study that just focuses only on one group. But in this study since the sample size of each individual ethnic group in the MOCDTBS was insufficient, we have opted to collect individuals from all ethnic groups to have enough sample size with sufficient power to detect the associations. For studies investigating three different ethnic groups like this study, each ethnic group must have enough sample size to be chosen to have the best possible result.
CONCLUSION

Within the limitation of this study, the conclusions following the objectives of the study are as below:

1. Descriptively, the distribution of the Arg/Arg and Arg/Gln when combined was found to be more in cases as compared to controls but when considered separately, only the Arg/Gln was seen higher among cases.

2. Either individually or combined there was no significant association between XRCC1 polymorphism and oral cancer risk. However, the odds ratio for combined genotypes (OR= 1.322) showed potential increased risk to oral cancer for the polymorphic types of XRCC1 codon399 (Arg/Gln,Gln/Gln). As individual genotypes, Arg/Gln indicated potential increased risk (OR: 1.108) and Gln/Gln indicated potential decreased risk (OR: 0.784) to have oral cancer.

3. There was no significant difference in distribution of XRCC1 genotypes between all ethnic groups respectively. However the distribution of Gln/Gln genotype has been seen highest among the Chinese in comparison to other ethnic groups.
This was the first study done in Malaysia to investigate whether XRCC1 polymorphism has any role or influence on oral cancer risk. The findings from this study have shown potential associations though were not significant perhaps due to small sample size. Therefore, it is strongly recommended for future investigation to be conducted on association between XRCC1 polymorphisms and oral cancer risk with a larger sample size to confirm our study findings.