POLYMORPHISMS OF CYTOCHROME P450 2E1 (CYP2E1) IN GASTROINTESTINAL CANCER

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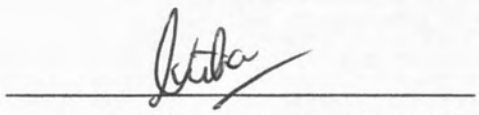

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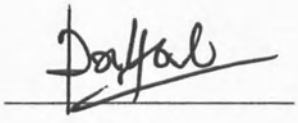

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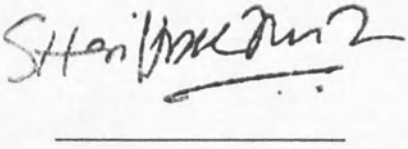

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ABSTRACT

Gastrointestinal cancer is a cancer that occurred in the digestive system. The cancer includes stomach cancer, liver cancer, pancreas cancer, esophageal cancer, colorectal cancer and anus cancer. This gastrointestinal cancer is known to be associated with the polymorphism of cytochrome P450 2E1 (CYP2E1) gene that are responsible in the oxidation of many low molecular weight procarcinogens and involved in the metabolism of ethanol and acetone. Moreover, the genetic polymorphism of CYP2E1 gene had been associated to the susceptibility to gastrointestinal cancer. Thus, the purpose of this study is to investigate the polymorphism of CYP2E1 gene and its association with gastrointestinal cancer phenotype. The study was conducted by using the 11 blood samples from the gastrointestinal cancer patients collected from the Queen Elizabeth Hospital. The DNA extraction was done by using the Qiagen Flexigene kit. PCR – RFLP was conducted to determine the genotypes in CYP2E1 using *Dra*I restriction enzyme. The PCR-RFLP was done twice in order to confirm the genotype analysis of CYP2E1 polymorphism. As a result, three genotypes were revealed which were the heterozygous (C/D), homozygous variant (C/C) and the homozygous wild type (D/D). From the statistical analysis, allele frequency for D allele (0.56) obtained higher compare to the C allele (0.44). Genotype frequency for heterozygous was 0.4925, homozygous wild type was 0.3136 and homozygous variant was 0.1936. These analysis shows that there was no clear correlation of CYP2E1 *Dra*I polymorphism and gastrointestinal cancer.

ABSTRAK

Kanser gastrousus adalah kanser yang berlaku di bahagian saluran pencernaan. Kanser in termasuk kaser perut, kanser hati, kanser pankreas, kanser esophagus, kanser kolorektal dan kanser dubur. Kanser gastrousus telah dikaitkan dengan polimofisme Gen sitokrom P450 2E1 yang bertanggunjawab dalam pengoksidaan prokarsinogen yang mempunyai jisim molekul yang rendah seperti benzene, styrene dan nitrosomines begitu juga dengan gen ini terlibat dalam metabolisma bagi etanol dan aseton. Genetik polimofisme bagi CYP2E1 gene telah dikaitkan dengan risiko kepada kanser gastrousus. Oleh itu objektif bagi kajian ini adalah untuk melihat hubungan polimorfisme bagi CYP2E1 gene dan kanser gastrosus. Kajian ini telah dilaksanakan dengan menggunakan 11 sampel daripada pesakit kanser gastrousus yang diperolehi dari Hospital Queen Elizabeth. Pengekstrakan DNA telah dilakukan dengan menggunakan Qiagen Flexigene kit. PCR-RFLP telah digunakan untuk mengenalpasti genotaip dalam CYP2E1 dengan menggunakan *Dra*I enzim pembatasan. Prosedur PCR telah dilakukan sebanyak dua kali untuk memastikan kewujudan DNA dalam sampel. Sebagai keputusan, tiga genotaip telah didedahkan iaitu heterozigos (C/D), homozigos varian (C/C) dan homozigos jenis liar (D/D). Daripada analisa statistic, alel frekuensi bagi alel D adalah 0.56, dan alel C analah 0.44. Manakala genotaip frekuensi bagi heterozigos adalah 0.4925, homozigos jenis liar dengan 0.3136 dan homozigos varian 0.1936 Daripada analisa, ini menunjukan tiada perkaitan yang jelas boleh dilihat bagi polimorfisme CYP2E1 *Dra* I gen dengan kanser gastrousus.

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LIST OF SYMBOLS

%	percentage
µl	microlitre
µg	microgram
ml	mililitre
mm	millimeter
kb	kilo bases
U	unit
°C	celcius
rpm	revolutions per minute
xg	centrifugal force
ng	nanogram
µM	micromole
mM	miliMole
s	second
min	minutes

CHAPTER 1

INTRODUCTION

1.1 Background

Cancer is a dangerous disease where cells tend to grow aggressively, invade the adjacent tissue and eventually spread to other part in the body. There are many types of cancer for examples, breast cancer, skin cancer, lung cancer, leukemia, bone cancer and so on. Among these cancers, gastrointestinal cancer includes cancer of the esophagus, stomach (gastric), liver, gall bladder and bile duct, small bowels (intestine), colorectal and anus.

In this study, the gastrointestinal cancer was investigated. Gastrointestinal cancer is a type of cancer that occurs in the organs of the digestive system. The digestive system consists of several organs such as the esophagus which is a tube where food will travel down, the stomach which is an organ that holds and digest food. As well as the small intestine which is a long tube that connects the

stomach to the large intestine. The waste are then passes out as a feces through the anus. It is a disease where malignant cancer grows anywhere from the esophagus to the anus of the digestive system.

According to the researches that have been conducted, there were changing pattern of gastrointestinal cancer in the Asian region. The highest incidence of gastric cancer (GCA) has been reported for Asia and it remains a very important cancer (Goh, 2007). Other than that, colorectal cancer also has become one of the most common cancers in many Asian countries. In recent years, Hong Kong, Korea, and Singapore have witnessed a steady rise in incidence in both males and females (Sung, 2004). In Malaysia, colorectal cancer are among the most common cancer while, gastric cancer is the second most common gastrointestinal cancer for both men and women (Goh *et al.*, 2007).

Like many other cancer, there are many factors that can be related causing the gastrointestinal cancer such as the intake of nitrosomide and nitrosomine compound in the diet which could contribute to the induction of colon tumors (Lijinsky, 2006). Moreover, the roles of alcohol consumption and overweight on risk of gastrointestinal cancer have become much clearer (Van den Brandt *et al.*, 2006). There were also studies that showed polymorphism of cytochrome P450 2E1 (CYP2E1) gene that can contribute to gastrointestinal cancer (Uematsu *et al.*, 1992). Particularly in intron 6 that is known to be highly polymorphic in the gene. CYP2E1 gene activates procarcinogens, organic solvents, and drugs,

converting them into carcinogenic products and potentiating the toxicity of solvents and drugs (Hatagima, 2002).

Since one of the factor that contribute to the gastrointestinal cancer is the polymorphism of cytochrome P450 2E1 (CYP2E1) gene, the correlation between the polymorphism of cytochrome P450 2E1 (CYP2E1) and gastrointestinal cancer is investigated by isolating the DNA from blood sample of the gastrointestinal cancer patients in Sabah. The polymorphism of P450 2E1 (CYP2E1) gene was analyzed by using the Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. The polymorphism was detected by using *Dra I* restriction enzyme.

1.2 Objectives

The objectives for this study are:

1. To isolate DNA from blood sample of gastrointestinal cancer patient in Sabah.

2. To analyze the cytochrome P450 2E1 *Dra* I in gastrointestinal cancer.

3. To investigate the cytochrome P450 2E1 *Dra* I polymorphism and its association with gastrointestinal cancer.

CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

Cancer can affect everyone, without recognizing its victim. Cancer is a dangerous disease but largely preventable. Cancer is also classified as one of the leading causes of death in the world with 12% of all death worldwide. According to World Health Organization statistic, More than 20 million persons around the world live with a diagnosis of cancer and more than half of new cancer cases occur in the developing countries (Nagoma, 2006). In 10 million new cancer cases each year, 4.7 million are in the more developed countries and nearly 5.5 million are in the less developed country (World Health Organization, 2002). It is estimated that in 2020 the number of new cases of cancer each year will grow to 15 million comparing 10 million in 2000.

2.2 Gastrointestinal cancer

Gastrointestinal cancer is the most common type of cancer worldwide but the incidence varies greatly from organ to organ in the digestive system (Waller et al., 2005). Gastrointestinal cancer is a malignant cancer that grows at the part of the digestive system. It includes the esophagus, stomach, liver, gallbladder, pancreas, small intestine, colorectal and anus region. Similar with other parts of the body these organs are made of many types of cells. Cells will continually divide but if the cells divide in uncontrolled way they can eventually form benign or malignant tumor.

The function of digestive system is to process the food. This process is called the digestion process. It takes food, digests and expels the remaining waste. Food passes through the gastrointestinal tract which is a long, hollow passageway that begins at the mouth and continues through the esophagus, the stomach, the small intestine, the colorectal and the anus (Wagman, 1987). The other organs such as the liver, the gall bladder and the pancreas take part in the digestion by releasing substances into the gastrointestinal tract that can help the digestion of many types of food substances. Below figure shows the anatomy of the digestive tract.

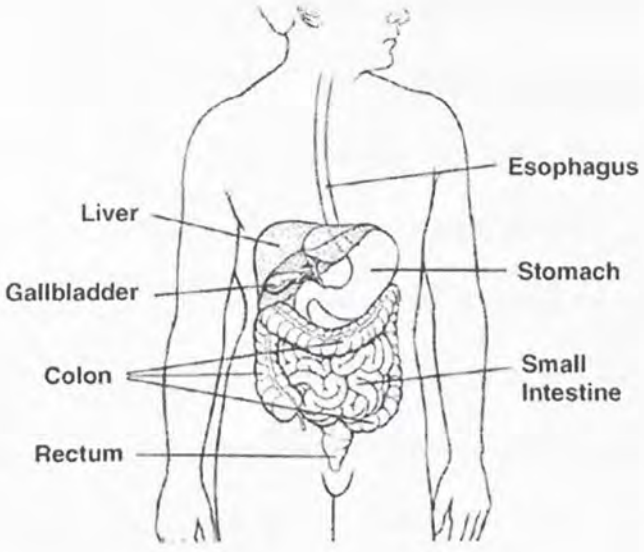

Figure 2.1 Anatomy of the gastrointestinal tract

(American Cancer Society, 2006)

Most cancer in the gastrointestinal tract starts from the glandular cells that will eventually develop into cancerous tumor of the adenocarcinoma. Other than glandular cells, there are also cell called squamous cell which are found mainly in the esophagus and the anus. Cancer that arises from this cell will eventually form into squamous cell carcinoma.

2.3 Types of gastrointestinal cancer

Gastrointestinal cancer is a cancer that affect the esophagus, stomach, liver, gallbladder and bile duct, pancreas, small intestinal, colorectal and anus. Each cancer arises at different part of the gastrointestinal tract with different symptoms, characteristic and occurrences.

Esophageal cancer is a cancer that occurred in the lining of esophagus and the gastroesophageal junction. There are mainly two types of esophageal cancer which are the squamous cell carcinoma that arise from the squamos cells and adenocarcinoma that grow in the glandular cells at lower part of esophagus. Most patients with esophageal cancer have dysphagia and odynophagia at the time of diagnosis (Enzinger and Mayer, 2003). Other common symptom for this cancer is weight loss. Cough, hoarseness and abdominal pain occurs less often compare to other symptoms (Enzinger & Mayer, 2003).

Stomach cancer is also known as gastric cancer. Adenocarcinoma is the most common stomach cancer. This cancer is difficult to diagnose at an early stage because there are no identifiying sign or symptoms (Walter, 1986). There are some symptoms such as indigestion that won't go away, weakness, bloated feeling after eating, vomiting or weight loss that might relate to stomach cancer.

Liver cancer is a cancer that arises from the tissue of the liver. The liver cells also known as hepatocytes that make up most of the liver tissue. Thus, most of the primary liver cancers arises from liver cells and is called hepatocellular cancer. About 25 – 30% of patients with hepatocellular cancer present with weight loss, malaise or anorexia (Oberfield *et al.*, 1989). Other than that abdominal pain is common in the cancer whereas, fever with unknown causes is not common symptoms.

Cancer of the bile ducts and cancer of the gall bladder have generally the same histological type. Over 90% Gallbladder cancer are adenomacarcinoma (Lazcano-Ponce *et al*, 2001) and more common compare to the bile duct cancer. Both cancers can present with similar symptoms such as pain in the right hypochondrium, jaundice, nausea and vomiting or severe weight loss.

Pancreatic cancer is a cancer that starts in the pancreas cells. The exact site of the cancer origin is unknown but it commonly starts to develop from the ducts that carry the pancreatic juice. The head of the pancreas is the primary site in about 75% of most cases (Raskin *et al*, 1961). Loss, abdominal or back pain and jaundice are the common symptoms while the less frequent symptoms are nausea, vomiting, diarrhea and aneroxia (Murr *et al*, 1994).

Small intestinal cancer is a rare cancer. There are different types of cancer can occur in the small intestine such as adenocarcinoma, sarcoma and carcinoid tumors. The symptoms for small intestine cancer are bloating, nausea and loss of appetite for the early stage. For the advance stage fatigue, weight loss and anemia are the common symptoms.

Colorectal cancer is a cancer that starts in the colon or rectum. This cancer begins as intramucosal ephitelial lesions arises form adenomatous polyps or glands (Feldman *et al.*, 2002). Most of colon colorectal cancers are characteristically adenocarcinoma. The common symptoms for this cancer are

stool with blood or mucus, constipation, diarrhea, abdominal pain and change in bowel habits.

Anal cancer is another rare form of cancer that affecting the anus. Cancer arising in the anal canal can be either keratinizing or non keratinizing which depend on the location. Most patients with this cancer present with rectal bleeding (Uronis and Bendell, 2007). Other common symptoms include rectal pain, changes in bowel habits, a lump located near the anus, anal discharge or pain in or around the anus.

2.4 Staging

Staging is the process after the diagnosis of cancer. It is to find out how far a cancer has invaded in the body. By knowing the spreading of these cancers we can determine the types of treatment necessary and estimate the rate of recovery from treatment and for survival. The staging of gastrointestinal cancer is based on the Tumor- Nodes- Metastasis (TNM) classification.

2.5 Epidemiology

Stomach cancer is one of the most common cancers in Asia (Goh *et al.*, 2007). However, the incidence rates in Asia are vary greatly from one country to another country and different ethnic groups. Age standardized incidence rates in India, Thailand and Philippines are among the lowest in the world and range from 15 to

100,000 per year (Goh, 2007) while, Chinese have the highest incidence rates. The stomach cancer also one of the most common cancers in Malaysia where about 1,400 Malaysian develop a cancer of this type each year. Among the Malaysian, Chinese race was a strong independent predictor of stomach cancer (Goh *et al.*, 2007) compare to Indian and Malay.

Moreover, colorectal cancer has been steadily increasing in Asia as well as Malaysia. The Japanese and the Singapore Chinese are reported to have highest incidence rate among the others in the world (Goh, 2007). In the United State pancreatic cancer is the fourth most frequent cause of cancer mortality while, in Japan it ranks as the fifth commonest cause of death from cancer (Lowenfels & Mainsonneuve, 2004). The incidence of liver cancer varies widely throughout the world, with high rates in sub-Sahara Africa, eastern and southeastern Asia, and Melanesia and a low incidence in Northern and Western Europe and the America (Srivatanakul *et al.*, 2004).

2.6 Cytochrome P450 2El (CYP2El)

Cytochrome P450 2E1 is a member of the cytochrome P450 familiy that encodes the enzyme N, N-dimethylnitrosamino-N-dimethylase, which catalyzes the oxidation of many low molecular weight procarcinogens like benzene, styrene and the nitrosomines as well as involved in the metabolism of ethanol and acetone (Hatagima, 2002). It is also essential for the metabolization of many medications such as important drugs. Thus, cytochrome P4502E1 plays an

important role in activation of procarcinogen, important drugs in medicine and organic solvent.

2.6.1 Cytochrome P450 2E1 pathway

In the methabolism of CYP2E1, the iron in the heme prosthetic group binds with oxygen which is eventually reduced by accepting an electron from nicotine amide-adenine dinucleotide phosphate (NADPH) (Feldman *et al.*, 2002). Then it will be bind into substrates such as drugs or toxins that will eventually result in the formation of reactive intermediate such as free radicals, electrophiles and reduced oxygen species (ROS).

Many toxic and carcinogenic compounds are not harmful to human in the parent form which they enter the body. However, metabolic activation by cytochrome P450 caused the compound to become reactive thus, exerting their harmful effects (Saarikoski *et al.*, 2005). These carcinogenic compounds include food additive and tobacco. The pathway of metabolic activation of the procarcinogen by cytochrome P450 2E1 can produce either reactive electrophile or stable hydroxylated compound (Tannock *et al.*, 2005).The reactive electrophile will then interact with negatively charged electron-rich group biological molecule such as DNA to form covalent adducts. If the DNA adducts are not repair before the next cycle of the DNA replication, it can cause mutation at the site. This condition will eventually activate the malignant transformation.

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