SINGLE NUCLEOTIDE POLYMORPHISM OF X-RAY REPAIR CROSS COMPLEMENTING 1 (XRCC1) GENE AT CODON 194 (ARG TO TRP) IN GASTROINTESTINAL CANCER

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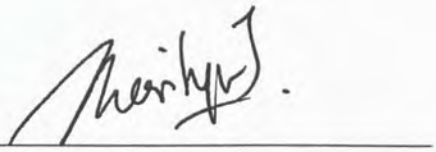

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ABSTRACT

This study focused on the polymorphisms in the X-Ray Cross Complementing 1 (XRCC1) gene in the gastrointestinal cancer. The main objective of this study is to investigate the effect of single nucleotide polymorphisms of XRCC1 at codon 194 (Arg → Trp) on gastrointestinal cancer. This study was conducted with a case-control study which includes 11 patients with gastrointestinal cancer from Queen Elizabeth Hospital and one healthy individual as a control case. There were four methods were done for the molecular analysis of the polymorphisms in the gene. The genomic DNA was extracted from the whole blood and was used to genotype XRCC1 Arg194Trp by using the polymerase chain reaction (PCR). PCR will amplify the region of DNA that contains the Arg194Trp site. The PCR products were digested using restriction enzyme, *Pvu*II. The resulting restriction products were then visualized on agarose gel electrophoresis. The result of this study showed that, the genotype frequency for the homozygote wild-type is 0.625 and the genotype frequency for the heterozygote and homozygote variants are 0.375 and 0.0 respectively. Therefore, the polymorphism of XRCC1 gene at codon 194 (Arg to Trp) affects the development of gastrointestinal cancer and contributes to a lower risk in the gastrointestinal cancer, in which the genotype frequency of the heterozygous variant was very low that is 0.375. Since the case-control study was very low in number, thus statistical analysis is could not be performed. However, the data were analyzed by determining the allele frequency of the genotypes. These suggested that, XRCC1 Arg194Trp genotype contribute to a lower risk of gastrointestinal cancer in the Sabah population.

ABSTRAK

Disertasi yang ditulis ini adalah mengenai projek yang memfokus kepada kajian polimorfisme dalam gen "XRCC1" dan kaitannya dalam kanser penghadaman. Objektif utama kajian ini adalah untuk menyiasat kesan genetik polimorfisme dalam "XRCC1" pada kodon 194 (Arg to Trp) dalam kanser pencernaan. Kajian ini dijalankan dengan kajian kes-kontrol dimana ia terdiri daripada 11 orang pesakit kanser penghadaman daripada Hospital Queen Elizabeth dan seorang individu yang sihat sebagai kes "control". Dalam disertasi ini, empat kaedah telah dijalankan untuk membuat analisis molekul terhadap polimorfisme dalam gen XRCC1. Genomik DNA yang telah dipencil daripada kesemua sampel darah akan digunakan untuk membentuk genotip XRCC1 Arg194Trp dengan menggunakan kaedah tindakbalas berantai polymerase. Dalam kaedah tindakbalas berantai polymerase, kawasan khas Arg194Trp akan direplikasi dalam bilangan yang banyak. Kemudian, DNA yang telah dihasilkan daripada kaedah tindakbalas berantai polymerase itu akan dipotong oleh enzim pemotong iaitu enzim *Pvu*II pada kawasan yang dikenalpasti oleh enzim itu. Akhirnya, produk pemotongan itu akan di analisis dalam agaros gel elektrophoresis. Keputusan bagi kajian ini adalah, frekuensi bagi homozigot "wild-type" adalah 0.625, manakala frekuensi heterozigot dan homozigot "variant" masing-masing adalah 0.375 dan 0.0. Ini menunjukkan bahawa polimorfisme dalam gen XRCC1 iaitu Arg194Trp menyumbang kepada perkembangan kanser penghadaman ini dan menyumbang kepada risiko rendah dalam kanser penghadaman. Memandangkan sampel bagi kajian ini adalah sedikit, analisis statistik tidak dapat dijalankan. Kesimpulannya, genotip XRCC1 Arg194Trp menyumbang kepada risiko rendah dalam pesakit kanser penghadaman dalam populasi Sabah ini.

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LIST OF SYMBOLS

SNPs	Single nucleotide polymorphisms
XRCC1	X-Ray Repair Cross Complementing 1
Arg	Arginine
Trp	Tryptophan
PCR	Polymerase Chain Reaction
µl	microlitre
%	percentage
°C	Celsius
S	second
min	minute
hr	hour
A_{260}	Absorbance at 260nm
A_{280}	Absorbance at 280nm
µg	microgram
g	gram
ml	milliliter
µg/ml	microgram per milliliter
mm	millimeter
U	Unit
rpm	revolutions per minute
xg	centrifugal force
nm	nanometer
ng	nanogram
µM	microMole
mM	miliMole
$MgCl_2$	magnesium chloride
dNTP	Deoxynucleotide triphosphates
A	Adenine
G	Guanine
C	Cytosine

CHAPTER 1

INTRODUCTION

1.1 Background of study

Gene is the unit of heredity in living organisms which encoded in a sequence of nucleotide monomers that make up a long strand of deoxyribonucleic acid (DNA). A particular gene can have multiple different forms, or alleles, which are defined by different sequences of DNA. The combinations of the alleles are known as the genotypes. Cells in the living organisms contain the hereditary information necessary for regulating cell functions and for transmitting information to the next generation of cells. A human cell has genetic material in the nucleus (the nuclear genome) and in the mitochondria (the mitochondrial genome). Cells are growing in a regulated manner. If mutation occurs on the gene, it sometimes causes either disease or cancer. Somehow, mutation in genes will affect the cell by changing the characteristic of the cell. The cells will display the traits of uncontrolled growth, invade other healthy cells, spread to other parts of the body via lymph or blood and destroy them. This type of cell is known as cancer cell. Cancer cells could also arise from polymorphisms in the gene.

Cancer is a disease which involves out-of-control growth or by an uncontrolled and spread of abnormal cells in part of the body. These cells accumulate and form tumors, which are in form of lumps that may compress, invade, and destroy. If cells break away from such a tumor, they can travel or spread through the blood stream or the lymph system to other areas of the body. The spread is called metastasis, which is also known as development of secondary tumors at a distance from a primary site of cancer. In their new location, the cancer cells continue growing. Different types of cancer vary in their rates of growth, patterns of spread, and responses to different types of treatment.

Gastrointestinal system which is also known as digestive tract contains organs that involve in digestion and absorption of the food we eat. The human digestive tracts are consists of esophagus, liver, stomach, gallbladder, pancreas, small intestine, large intestine, rectum and anus. The gastrointestinal tract can be inflamed and irritable for unknown reasons, can contract in spasms, and develops pouches that become infected, or can develop swelled veins that can cause pain and bleeding. And of course, gastrointestinal tract is a primary area for cancers to develop and hide.

Gastrointestinal cancers are malignant tumors that occur in the digestive system of a human. Malignant tumors are cancerous in which the cancer cells can spread to nearby healthy cells and destroy them and they can invade other parts of the body. Cancerous cells in the digestive system can spread to the lymph glands that are located nearby. Gastrointestinal cancer is also a general term that sometimes used for a group of different cancers can occur in the lower gastrointestinal tract including colon or rectal cancer, liver cancer and pancreatic cancer (Altman & Sarg, 1992).

According to author Morra Marion and Potts Eve of *CHOICES,* the cancers that usually develop in the gastrointestinal tract are esophageal cancer, stomach cancer, liver cancer, pancreas cancer, gallbladder cancer, small bowel cancer, large bowel cancer and anal cancer.

The general symptoms of gastrointestinal cancer are changes in bowel habits, such as constipation or diarrhea, produce a very dark or mahogany red or bright red blood in or on the stool, has an abdominal discomfort, gas pains or cramps, constant indigestion or heartburn, persistent narrowing of the stools, urgent and painful need to have a bowel movement (discharge faeces from the body), feeling of incomplete emptying following bowel movement, unexplained weight loss, anemia, vomiting and a need for frequent urination and a feeling of lump or mass in abdomen.

The gastrointestinal cancer is usually diagnosed by physical examination, digital rectal examination, endoscopy, direct visual inspection (sigmoidoscopy and/or colonoscopy), barium swallow, X-Ray examination of large bowel called lower gastrointestinal cancer series, fecal occult blood stool test, intravenous pyelogram (IVP) and carcinoembroyonic antigen (CEA) blood test.

All common gastrointestinal cancers are staged using the T (tumor size and extent), N (number of lymph nodes involved with cancer) and M (distant metastasis to other organs) staging system as described by the American Joint Committee on Cancer (Greene *et al.,* 2002). The TNM staging is used as a guide for prognosis for the patient, and to assist medical professionals in making treatment decisions. It is often translated into Stages I to IV utilizing the TNM data. Patients with early stage

disease have a better prognosis than those with locally advanced (T3 of above) or metastatic disease (designated as an M1 or stage IV). Radiological exams are used in all these cancers to assist in staging of the cancer, and determine the surgical resectability of cancerous tumors.

The aetiology of the gastrointestinal cancers are environmental factor such as smoking and alcohol consumptions, diet such as diet high in salted, smoked or preserved foods, and age factor. Aside from the environmental, diet and age factors, other risk factor of gastrointestinal cancer is the genetic factor in which the cancer is inherited through family members based on their family history. It is caused mainly by heritable germ line mutation which arose by polymorphisms in certain genes and other genes appeared to be associated with the cancers. Although genetics plays a large role in determining the appearance and behavior of organisms, it is the interaction of genetics with the environment an organism experiences that determines the ultimate outcome. For example, while genes play a role in determining a person's height, the nutrition and health that person experiences in childhood also have a large effect. Apart from it, bacteria infection such as *Helicobacter Pylori* bacteria causes infection on the stomach and *Salmonella typhi* bacterial infections on the gallbladder.

People have been taken the digestive system in their bodies for granted. After all, without thinking about it, the mechanisms inside their bodies have been working badly or without instructions for years. Nowadays, people are more willing to eat many delicious foods without taking care of the circumstances to their bodies. Apart from it, people find it to be unpleasant for digestive system for conversation. This may explain why it is easy to ignore the warning signals or early symptoms of the cancers.

This phenomenon has contributed to the high number of cases of gastrointestinal cancer and it has become one of the popular cancer diseases in the world.

In Malaysia, cancer is one of the major health problems. Cancer of the rectum is among the top four cancers in Malays, Chinese males and Indian males (Chan *et al.*, 1994). According to the report of the National Cancer Registry of Malaysia 2003, colon cancer ranks as the third most common cancer for both men and women, with a higher rank than gastric cancer (Lim & Halimah, 2004). According to the study done by Goh *et al.*, (2007), bivariate analysis showed that age more than 65 years and Malay and Chinese race compared with Indian were significantly associated with colorectal cancer in Malaysia. Colorectal cancer is also a common solid tumour in Malaysia. The Chinese race in Malaysia was identified as a strong independent predictor of gastric cancer and the *H. pylori* was also a significant risk factor for the development of the gastric cancer (Goh *et al.,* 2007). As the number of cases of gastrointestinal cancers increase, there were many genes contributes to the development of the gastrointestinal cancer.

Genetic susceptibility may play an important role in risk of gastrointestinal cancers. The genetic susceptibility may result from inherited polymorphisms in certain genes and other genes appeared to be associated with the cancers. Ultimately, all polymorphisms arose as mutations. Polymorphisms are discontinuous variation in a single population. Another term that is important is the single nucleotide polymorphisms in which mutation occur by a single base substitution in the gene. It alters the functions of genes and could contribute to the development of many diseases or cancer. The predominant mechanisms for the cancers featured are impairment of a

DNA repair pathway, the transformation of a normal gene into an oncogene and the malfunction of a tumor suppressor gene.

According to Vogelstein & Kinzler (2004), examples of tumor suppressor genes that contribute to the gastrointestinal cancers are Adenomatous Polyposis (APC), Axis Inhibitor 2 (AXIN2), tumor protein (p53), Serine/Threonine protein Kinase (STK11), Bone morphogenetic protein receptor (BMPR1A) and Mother against Decapentaplegic Homolog 4 (SMAD4). For the DNA repair genes, the polymorphisms of genes that contribute to the hereditary factors to the gastrointestinal cancer are Muts, *E. Coli* Homolog of 2 and 6 (MSH2 and MSH6). Besides, other genes that contribute to the gastrointestinal cancers are Glutathione *S*-transferases (GST) P1 (Lecomte *et a*l., 2006), member of the cytochrome P450 (CYP2A6), X-ray repair cross complementing (XRCC2, XRCC3 gene) (Yeh *et al*., 2005) and apurinic/ apyrimidinic endonuclease 1 (APE1) (Jiao *et al.,* 2006).

Since many genetic polymorphisms of the genes have been contributed to the gastrointestinal cancers, approach has been done in this study in which will investigate the single nucleotide polymorphisms of the X-ray repair cross complementing 1 (XRCC1) gene in the development of the gastrointestinal cancers. XRRC1 is a scaffold protein for both single-strand break repair and base excision repair activities. XRCC1 is directly associated with DNA polymerase beta, poly (ADP-ribose) polymerase (PARP) and DNA ligase III to facilitate base excision repair (BER) (Caldecott *et al*., 1996).

Shen *et al*. (1998), has reported that the polymorphisms of the XRCC1 gene have been identified at the conserved sequences and resulting in amino acid substitutions. The polymorphisms include single nucleotide polymorphisms at codon 194 (Arg to Trp), 399 (Arg to Gln) and 280 (Arg to His). However this study will focus on the polymorphism of the XRRC1 gene on the single nucleotide polymorphism at the codon 194, Arginine to Tryptophan (Arg to Trp). Codon is sequence of three adjacent nucleotides that designates a specific amino acid and site for transcription. The polymorphism of Arg194Trp occur on exon 6 at base 26304, codon that specifies for Arginine amino acid has changed to Tryptophan, in which base C substitutes to T.

This study was conducted to know whether the single nucleotide polymorphism of Arg194Trp on the XRRC1 gene has the role to potential develop the gastrointestinal cancers among the population in the world, and more significantly in the Sabah population. The methodologies used in conducting this study were extraction of DNA from the blood samples of gastrointestinal cancers patients as well as control, amplification of the region of the XRCC1 gene containing the Arg194Trp site from the extracted DNA and restriction enzyme digestion with enzyme *Pvu*II. Finally, the resulting restriction products will be observed on agarose gel electrophoresis. Statistical analysis was then performed to know the proportions or frequencies of the allele Arg and Trp and the genotypes frequencies in the gastrointestinal cancer patients.

Through the finding results on this study, it can identify whether the polymorphism of XRCC1 gene are associated with the high risk or lower risk of the gastrointestinal cancers patients in the Sabah population. Besides, through this study, one can learn more about gastrointestinal cancers, the symptoms and how to diagnose gastrointestinal cancers and can study the functions of the genes by applying the techniques in molecular analysis.

1.2 Objectives

The objectives of this study are;

1. To isolate the DNA from the blood samples of gastrointestinal cancer patients in Sabah and also healthy individual as control case.
2. To amplify the region of XRCC1 containing the Arg194Trp site from the extracted DNA.
3. To study the polymorphisms of DNA repair gene X-ray Repair Cross Complementing 1 (XRCC1) in gastrointestinal cancer patients in the population of Sabah.
4. To identify whether the polymorphism in the XRCC1 gene by means of polymorphism at the codon 194 (Arg to Trp) contribute to a higher risk or lower risk in the development of gastrointestinal cancer.

CHAPTER 2

LITERATURE REVIEW

2.1 Gastrointestinal Tract

Gastrointestinal tract runs from the mouth to the anus. It is like a long tube whose cavity or lumen is surrounded by a wall that absorbs nutrients. The ability to absorb is heightened by the wall's surface of ridges and valleys, which expand the area that comes in contact with nutrients. Gastrointestinal tract is vulnerable to what we eat and to our daily stresses and that contains organs that involved in digestion and absorption of the food we eat. Gastrointestinal tract is consists of organs such as esophagus, stomach, liver, pancreas, gallbladder, small and large intestines/bowel and anus. The food we eaten will passed sequentially through the esophagus, the stomach, small intestine and finally the large intestine and rectum. The wastes go out anus. Each of these organs has specialized function in digestion. The liver, gallbladder and pancreas are organs that help digest food. The appendix does not affect digestion. Figure 1.1 shows the figure of gastrointestinal tract of human.

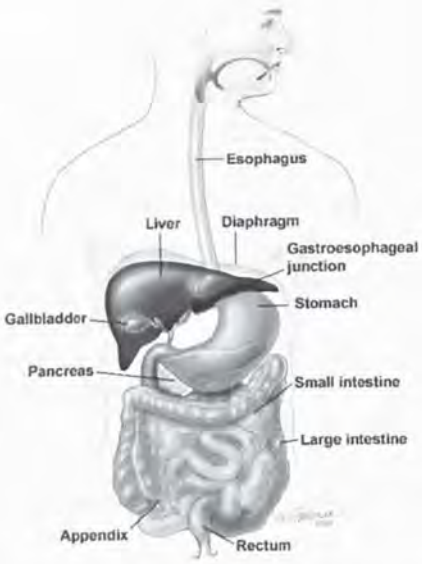

Figure 2.1: Gastrointestinal tract of human

Source: Maxwell Blum resource room Massachusetts General Hospital, Boston, MA.

2.2 Gastrointestinal Cancers

Esophageal cancer occurs when cells in the lining of the esophagus grow uncontrollably and form tumors that can invade normal tissues and spread to other parts of the body. Esophagus is a foodpipe that is about 10 inches long and a hollow muscular tube functions as carrying food and liquids. Esophageal cancer is rarely happen compared with the other cancers of the digestive tracts and one of the least curable and cause death cancer. This cancer commonly occurs in men than women and blacks are more potential to develop three to four times more often than the whites. It occurs most often between the ages of 50 and 70. The most common symptom of esophageal cancer is dysphagia in which a pain or difficulty in swallowing solids or liquids. Other than that are severe losses of weight, pain in throat

or back, behind breastbone or shoulder blades, hoarseness, cough, vomiting, coughing up blood, indigestion and heartburn and pain in the chest that is not due to eating.

Gallbladder cancer is a rare cancer and is less often seen cancer that invades other parts of the digestive system. Gallbladder is a small sac-shaped organ beneath the liver in which it stores the bile. Bile is a greenish liquid substance produced by the cells of the liver that helps in the emulsification causing the fats to accumulate into droplets which can be easily absorbed in the small intestine. It also aids in the absorption of fat soluble vitamins, such as vitamins A, D, E and K. Gallbladder cancer occurs in women three times as often it does in men. It also develops among elderly people. The common symptom of this cancer is jaundice which is yellowing of the skin due to an excess of bile pigments in the blood, loss of appetite, weight loss and pain in the abdomen especially on the right under the ribcage. However, people often have no symptoms.

Liver is one of the largest organs in the body has two parts that are large right lobe and smaller left lobe. It performs many important complex functions including break down worn-out red blood cells and convert them into bile which assists digestion, regulates the level of many hormones, store sugar, clearing toxins from the blood, metabolize drugs and making blood protein. The cancer that arises from the liver is known as hepatocellular carcinoma which is the primary liver cancers. Men are likely to get twice as likely as women and this cancer often attack people who are over age of 60. Symptoms of liver cancer are discomfort in the upper abdomen on the right side which becomes more acute with deep breathing, a hard lump just below the rib cage on the right side, lack of appetite, pain around right shoulder blade,

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