GENETIC POLYMORPHISMS OF PROINFLAMMATORY (IL-1β) CYTOKINES IN MALAY MALE DRUG ABUSERS WITH HEPATITIS C INFECTION

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by

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LIST OF SYMBOLS

α	Alpha
β	Beta
γ	Gamma
χ	Chi
λ	Lambda
μ	Micro
°C	Degree Celsius
rpm	Revolution per minute
%	Percentage
U/L	Unit per liter
g	Gram
L	Liter
mL	Milliliter
μL	Microliter
mg/mL	Milligram per milliliter
ng/μL	Nanogram per microliter
min	Minute
s	Second
kg	Kilogram
&	And
pmol	Picomole
mM	Millimolar
U/μL	Unit per microliter
μM	Micromolar

μmol/L   Micromolar per liter

xg   Relative centrifugal force

LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate transaminase
GGT	Gamma-glutamyl transpeptidase
KCl	Potassium chloride
$MgCl_2$	Magnesium chloride
cDNA	Complementary deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphates
dsRNA	double strand ribonucleic acid
ssRNA	single strand ribonucleic acid
CNV	Copy number variation
CTL	Cyto T lymphocyte
DNA	Deoxyribonucleic acid
EASL	European Association for the Study of Liver
EAS	East Assia
GWAS	Genome-wide association studies
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HWE	Hardy-Weinberg Equilibrium
IFN	Interferon
IL	Interleukin
NGS	Next generation sequencing

NK	Natural killer
PCR	Polymerase chain reaction
PMN	polymorphonuclear leukocytesSTR
PWID	Person with injection drug
qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative reverse transcriptase polymerase chain reaction
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
RT-PCR	Reverse transcriptase polymerase chain reaction
SNP	Single nucleotide polymorphism
TBE	Tris-Borate E
TMD	Transmembrane domain
TRAPS	TNF receptor-associated periodic syndrome
WHO	World Health Organisation

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POLIMORFISME GENETIK SITOKIN PRO-RADANG (IL-1B) DALAM KALANGAN PENYALAHGUNA DADAH LELAKI MELAYU DENGAN JANGKITAN HEPATITIS C

ABSTRAK

Jangkitan virus hepatitis C (HCV) kerap mengakibatkan keadaan hati seperti fibrosis, sirosis, dan karsinoma hepatoselular, justeru menimbulkan risiko kepada kesihatan masyarakat umum. Tambahan pula, kejadian penularan jangkitan HCV membimbangkan di kalangan penyalahguna dadah. Pengantara keradangan antara sel, sitokin, adalah hasil sampingan dalam kawalan virus dan kerosakan hati yang disebabkan oleh jangkitan virus hepatitis C. IL-1β, khususnya, adalah sitokin proinflamasi yang dirembeskan semasa tindak balas imun semula jadi dan adaptif yang penting dalam penyakit keradangan kronik. Banyak kajian telah melaporkan perhubungan sitokin ini dan kecenderungan mendapat jangkitan HCV kronik. Oleh itu, tujuan kajian adalah untuk menyiasat peranan polimorfisme IL-1β (rs16944 dan rs1143634) berhubung dengan kecenderungan mendapat jangkitan hepatitis C kronik. Seramai 48 orang penyalahguna dadah terdiri daripada lelaki Melayu terlibat dalam kajian kes-kawalan retrospektif ini. Genomik DNA diekstrak daripada sampel darah kemudian dianalisis melalui *polymerase chain reaction- restriction fragment length polymorphism* (PCR-RFLP) untuk polimorfisme IL-1β (rs16944) dan IL-1β (rs1143634). Analisis IL-1β (rs1143634) menunjukkan bahawa genotip CC adalah terbanyak dalam kedua-dua kumpulan kes dan kumpulan kawalan. Walau bagaimanapun, tiada signifikan statistik dicapai. Penyelidikan ini menyimpulkan bahawa tiada perhubungan antara polimorfisme (rs16944 dan rs1143634) dan

kecenderungan mendapat jangkitan hepatitis C dalam kalangan penyalahguna dadah

lelaki Melayu.

GENETIC POLYMORPHISMS OF PROINFLAMMATORY (IL-1B) CYTOKINES IN MALAY MALE DRUG ABUSERS WITH HEPATITIS C INFECTION

ABSTRACT

The hepatitis C virus (HCV) infection frequently results in liver conditions such fibrosis, cirrhosis, and hepatocellular carcinoma, therefore, it poses a serious risk to the general public health. Furthermore, the incidence of HCV infection transmission is concerning among drug abusers. The intercellular mediators of inflammation, cytokines, are byproduct in viral control and the liver damage brought on by hepatitis C virus infection. IL-1β, particularly, is a proinflammatory cytokine that is secreted during the innate and adaptive immune response that is pivotal in chronic inflammatory diseases. Studies have reported the association of this cytokine and susceptibility of chronic HCV infection. Therefore, the purpose of the study was to investigate the role of IL-1β polymorphisms (rs16944 and rs1143634) in regard to chronic hepatitis C infection susceptibility. A total of 48 Malay male drug abuser were involved in this case-control retrospective study. The genomic DNA was extracted from whole blood sample then analyzed through polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for the IL-1β rs16944) and IL-1β (rs1143634) polymorphisms. The analysis of IL-1β (rs1143634) showed that CC genotype was common in both case and control group. However, no statistical significance was achieved. This research concluded that there was no association between the polymorphisms (rs16944 and rs1143634) and susceptibility to hepatitis C infection among Malay male drug abuser.

CHAPTER 1

INTRODUCTION

1.1 Background

Hepatitis C virus (HCV) infection is recognised as a major public health issue globally as of 2015. It was estimated that 71 million people were diagnosed with chronic infection. Currently, the number stands at 58 million chronically infected individuals (WHO, 2022). Although the decrease in number of chronic infections is an optimistic insight, nevertheless, it does not extricate the disease from the severity of its infection. According to WHO (2022), in 2019, about 290000 deaths were reported due to HCV infection mainly from the progression of chronic infection to cirrhosis and hepatocellular carcinoma (HCC). Purportedly, 20% of persistent HCV infection led to the aforementioned diseases (Axley et al., 2018; Chen & Morgan, 2006). Moreover, 80% of acute HCV infections are asymptomatic making it hard to diagnose, abetting latent infection transmission and progression to chronic state (. In recent years, extrahepatic and partly extrahepatic manifestations have been reported in association with chronic HCV infection. These manifestations include cryoglobulinemia, lymphoma, glomerulonephritis, disturbances in lipid metabolism, and insulin resistance. These findings indicate that chronic HCV infection is a disease with multiple facets (Kuna et al., 2019; Koike, 2016).

Hepatitis C is a bloodborne liver infection disease. Injecting hazardous parenteral drugs, engaging in risky sexual activity and receiving a blood transfusion are the three most common ways that the HCV is spread. Patients, healthcare workers and drug abusers are the groups that are most at risk of developing the sickness given

these circumstances. As members of the population that fall within the category of being at an increased risk of infection, drug abusers have been singled out as a particular focus of attention in recent research studies (McCance-Katz & Valdiserri, 2015). Hazardous acts such as illicit drug use and sharing of contaminated syringes escalate the transmission of the disease.

Hepatitis C infection is endemic globally and the prevalence of the disease varies geographically (Perumalswami & Klein, 2014; Messina et al., 2015). The revision of hepatitis C virus infection classification made by Smith et al. in 2013 determined 7 major genotypes and 67 subtypes based on phylogenetic and sequencing analysis. An additional major genotype was found; updating the categories from the previous 6 major genotypes made in 1993 (Simmonds et al., 1993). Studies on the global distribution and prevalence of HCV genotypes shows diverse genotypes of 1, 2, 3 and 6 prevalence scattered across the Southeast Asia countries (Irekeola et al., 2021; Messina et al., 2015). The southeast Asia region has a particularly high prevalence of HCV genotype 6 within a 94.6 million infected individuals (Doan, 2018). In Malaysia context, several studies on the genotype prevalence stated HCV genotype 3 as the most frequent followed by genotype 1 (Ho et al., 2015; Mohamed et al., 2013).

In 2017, it was reported that the HCV infection had a 9.54 over 100 000 incidence rates, while the death rate was 0.29 per 100 000 people (Ministry of Health Malaysia [MOHM], 2020). This incidence rate is a concern to the Malaysian health system considering the association HCV infection with cardiovascular disease. Referring to the statistic report from the Department of Statistic Malaysia which states

ischemic heart diseases being the principal cause of death in the country, it is plausible the HCV infection is one of the major risk factors of cardiovascular disease. Previous studies have shown that HCV infection is a putative risk factor of cardiovascular artery disease (Cacoub, 2019).

Viral proteins and double-stranded RNA (dsRNA) from HCV cause the production of proinflammatory cytokines (Nishitsuji et al., 2013). During the early stages of an infection, the intricate cytokine network enables the formation of an immune response that is both innate and adaptive in a manner that is coordinated and efficient. The outcome of a viral infection is decided by the interaction between the host capacity to generate powerful antiviral responses and the viral mechanisms that neutralize such responses (Fallahi et al., 2012). It is impossible to place an adequate amount of emphasis on the significance of proinflammatory cytokines such as IL-1 when it comes to the regulation of the immune response. This is because many factors may affect how the immune system work especially when infected by infectious disease such as HCV infection. Addressing the genetic diversity of the host during the latent infection is one way to ascertain the significance association between the working behavior of cytokines.

The functional SNPs of IL-1 have diverse distribution among major population such as America, Europe, South Asia, Africa and East Asian. Studies discovered that IL-1 expressions and polymorphisms may worsen diseases condition (Nelson et al., 2016; Murphy et al., 2015; Kasprzack et al., 2004). Hence, there is a growing need to understand the role that SNPs play in the progression of chronic hepatitis C infection as more evidence of the relationship between SNPs and the advancement of diseases,

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particularly those diseases involving inflammation, is brought to light. Genotyping and SNP analysis provide information that can be used to build genome-based knowledge about an individual susceptibility to a variety of common diseases, produce safer and more effective personalized diets and treatments for patients, and understand the processes of evolution (Shastry, 2007). The major goal of this study is to investigate the role of IL-1 gene polymorphisms (rs16944 and rs1143634) in relation to hepatitis C infection in male drug abusers who are of Malay descent.

1.2 Problem Statement

The number of hepatitis C infection in Malaysia pose a concern to the general healthcare. The spread of the infectious disease is rampant amongst individuals with history of drug abuse. About 59% of HCV infected Malaysia patients known to have history of drug injection (Raihan, 2016). In addition, many studies conducted by local researchers found that person with drug injection (PWID) elevate the odds of being infected (Abdilah et al., 2020; Suan et al., 2019; Noh et al., 2019). Furthermore, drug abusers are evidently susceptible to developing chronic HCV infection due to liver failure from drug abuse and as individuals with high risk of infection through syringes sharing. As more evidence of genetic polymorphisms effect in the progression of HCV infection found substantial, there is a need for better clarifications among Malaysian drug abuser population.

The role of IL-1 as proinflammatory cytokine in the immune response network is diverse and pivotal. Evaluating the relationship between allelic and genotypic frequency of IL-1 gene such as -511 (rs16944) and +3954 (rs1143634) will provide an insight into the effect of respective polymorphisms in susceptibility and progression of HCV infection. Genotyping of aforementioned SNPs enable the identification of common polymorphisms present in Malay male drug abuser. Besides, more scientific evidence of local context can be contributed for future studies in this regard. In summary, it is suggested that there is an association between genetic polymorphisms of IL-1β and its role in the progression of HCV infections among Malay male drug abusers. Thus, the study provides perception into the effect of these particular SNPs of the IL-1β gene in Malay male drug abusers.

1.3   Objectives

1.3.1   General objective

To determine the genetic polymorphisms of pro-inflammatory (IL-1) cytokines in Malay male drug abuser with hepatitis C infection.

1.3.2   Specific objectives

There are several specific objectives to be achieved in the study which are:

i.   To perform genotyping of single nucleotide polymorphisms in interleukin-1 (IL-1β) cytokine (rs16944, rs1143634) using polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) in blood DNA from Malay male drug abusers.

ii.   To determine the allelic and genotype frequencies of IL-1β (rs16944) and IL-1β (rs1143634).

iii.   To assess the association between hepatitis C virus infection susceptibility and IL-1β genetic polymorphisms in Malay male drug abuser.

1.4　Overview of the study

The flowchart of the study overview is shown as in Figure 1.1 below:

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│              Literature Review               │
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                      ▼
┌─────────────────────────────────────────────┐
│               DNA Extraction                 │
└─────────────────────────────────────────────┘
                      ▼
┌─────────────────────────────────────────────┐
│              Data Collection                 │
├──────────────────────┬──────────────────────┤
│       PCR-RFLP        │   Gel Electrophoresis│
└──────────────────────┴──────────────────────┘
                      ▼
┌─────────────────────────────────────────────┐
│                Data Analysis                 │
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│               Final Reporting                │
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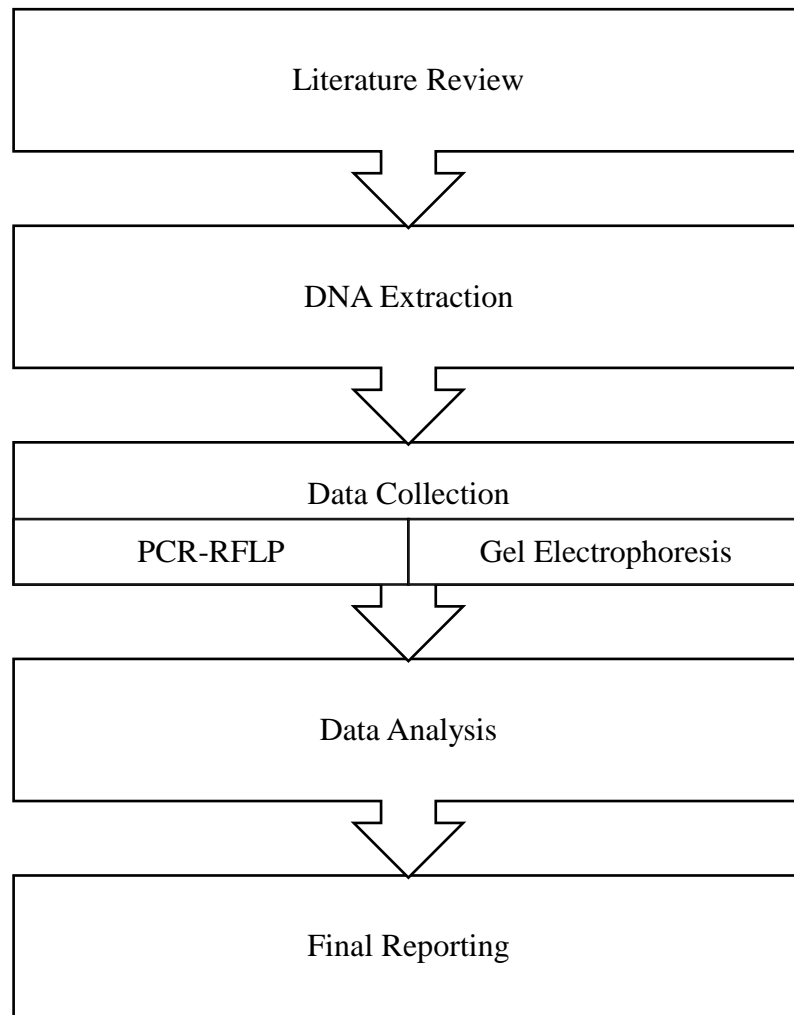
Figure 1.1　Flowchart of the study

CHAPTER 2

LITERATURE REVIEW

2.1   Hepatitis

Hepatitis is a medical disorder in which the liver becomes inflamed as a result of exposure to hepatotropic viruses, non-hepatotropic viruses, alcohol, drug misuse, an autoimmune condition, or other factors. As a result, this illness might be placed in one of several categories depending on the primary factor that led to the development of hepatitis. In particular, viral hepatitis has become a global problem since it is linked to a large morbidity and mortality rate in the community as well as a burden on the healthcare system. It was claimed to be responsible for millions of fatalities each year, which places it on par with other infectious diseases such as the Human Immunodeficiency Virus (HIV) and tuberculosis (WHO, 2016). According to the World Health Organization (WHO), which published its findings in the year 2022, there are already 325 million people infected with chronic hepatitis around the world.

Hepatitis caused by viruses can be further subdivided into five primary virus strains, which are designated by the letters A, B, C, D, and E. These viruses are distinct from one another in several ways, including the manner in which they are transmitted, the populations who are susceptible to them, and the health consequences they cause. In 1973, R.H. Purcell and a collaborator were able to identify the hepatitis A virus (HAV) from a stool sample using immunological electron microscopy (IEM). Before this occurrence, the hepatitis B virus, sometimes known as HBV, was discovered by B. Bloomberg and colleagues in the earlier part of the 1960s. This virus represents a significant threat to the health of the world population in the modern era because it is

responsible for between 500,000 and 1.2 million deaths each year, placing it as the tenth largest cause of death overall (Blum, 2019). Then in the year 1989, M. Houghton and some of his fellow researchers discovered the hepatitis C virus (HCV). In the wake of the discovery of HCV, rapid advancements have been made in the serology and molecular diagnostic of HCV assay system. These advancements have included genotyping of the strain. It was not until 1977 and 1983, respectively, that researchers identified the hepatitis D virus (HDV) and the hepatitis E virus (HEV).

2.2 Hepatitis C Virus (HCV)

Choo et al. made the discovery of HCV in the late 1980s by using cloning as their method of investigation (1989). The World Health Organization (WHO) estimates that there are currently over 58 million people living with chronic hepatitis C. Every year, there are roughly 1.5 million new HCV infections, bringing the total number to approximately 58 million. WHO estimates that in 2019, 29 000 persons lost their lives to cirrhosis and hepatocellular carcinoma as a direct consequence of the advancement of HCV infection.

The natural history of HCV infection describes two different types of persistence that can occur after infection: acute and chronic. According to Figure 2.1, the first six months following exposure to the virus are considered to be the incubation period for acute HCV infections. Spontaneous clearance of serum viral load is estimated to be around 15% to 30% (Heim, 2013; Thomson et al., 2010; Micallef et al., 2006). Other studies have reported that the percentage can reach up to 40% (Kong et al., 2014; Ayoub et al., 2018; Westbrook & Dusheiko, 2014). This is equivalent to stating that 60% to 80% of the viral load persist in the systemic circulation which