UNIVERSITI PUTRA MALAYSIA

ASSOCIATION OF HUMAN LEUKOCYTE ANTIGEN WITH PRECURSOR-B ACUTE LYMPHOBLASTIC LEUKAEMIA (PRE-B ALL) IN MALAYS

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By

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Acute Lymphoblastic Leukaemia (ALL) is one of the leukaemia subdivisions and is a malignant disorder of lymphoid progenitor cells originating from the marrow affecting both children and adults. Pathogenesis and epidemiologic studies of leukaemia in some population strongly suggest that this disease has an inherited basis and described an increase in allele frequency of HLA antigens that contribute as one of the risk factors. Thus, polymorphisms and different HLA genetic make-up in different ethnic groups make it crucial to study the disease in each population. Malays make up the largest population in Malaysia and the incidence of lymphoid leukaemia has also been reported to be highest in this group. It is hypothesised that there is an increased frequency of certain Class II (HLA-DRB1) alleles and also increase in levels of soluble HLA-DRB1 in ALL patients. The objectives of this study were to identify the HLA-DRB1 alleles associated with ALL patients of Malay ethnicity in Malaysia, and further sequence the identified risk alleles, and determine the soluble HLA levels in plasma in those patients compared to the healthy population. HLA-DNA Typing Class II was performed in pre-B ALL patients (n=42) by PCR-SSO (Polymerase chain reaction, sequence-specific oligonucleotides) and the data of HLA allele genotypes were compared with available data from healthy Malay population (n=1445). Furthermore, the plasma levels of soluble HLA class II (sHLA-DRB1) in patients (n=30) and controls (n=31) were determined by sandwich Enzyme-linked immunosorbent assay (ELISA). DNA sequencing of specific HLA-DRB1 alleles for the risk and associated alleles was also carried out on exon 2 by Sequence based typing (SBT) approach. Results show that there were higher allelic distribution of specific HLA-DRB1 alleles; HLA-DRB1*03 (P value=0.001, OR=2.92, 95% CI=1.47-5.80) and also –DRB1*16 (P value=0.001, OR=2.76, 95% CI=1.30-5.87) observed among Malay pre-B ALL patients. On the other hand, significant decrease in HLA-DRB1*07 (P value=0.032, OR=0.47, 95% CI=0.17-1.28) and -DRB1*12 (P value=0.008, OR=0.59, CI=0.33-1.03) were seen in these patients, would suggest protective potential for the disease. Further analysis by SBT was able to clarify the resolution of risk alleles, which were HLA-DRB1*03:01:01 and HLA-DRB1*16:02:01, respectively. Polymorphisms were observed between DNA sequences of polymorphic exon 2 of patient and control groups for –DRB1*03

but not –DRB1*16. An increase in levels of soluble HLA-DRB1 in plasma also was detected in the ALL patients (0.260 ± 0.057 µg/mL) compared to normal individuals (0.051 ± 0.007 µg/mL) with p value of 0.001. The results obtained support the earlier hypothesis and suggest that HLA Class II antigens are the susceptible genes and also as possible biomarker for ALL. This investigation may contribute to aetiology and pathogenesis studies on ALL and identify HLA as potential genetic risk markers or as cancer biomarkers, and also as reference for future studies.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

HUBUNGAN ANTARA ANTIGEN LEUKOSIT MANUSIA DAN LEUKEMIA LIMFOBLASTIK AKUT, PRE-B (PRE-B ALL) DI KALANGAN MELAYU

Oleh

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Leukemia limfoblastik akut (ALL) adalah salah satu sub-kategori leukemia dan merupakan penyakit malignan sel-sel leluhur limfoid yang berasal dari sumsum tulang dan memberi kesan kepada kanak-kanak dan juga dewasa. Kajian patogenesis dan epidemiologi leukemia di dalam populasi yang berbeza mencadangkan bahawa faktor utama penyakit tersebut adalah disebabkan oleh keturunan yang mana disifatkan oleh peningkatan dalam kekerapan alel HLA antigen yang menyumbang kepada salah satu faktor risiko. Oleh itu, polimorfisme dan maklumat genetik HLA yang berbeza di dalam etnik dan penduduk berlainan menjadikannya penting untuk kajian penyakit ini khasnya di dalam satu-satu bangsa yang sama, seperti mana yang ditunjukkan di dalam laporan, bahawa insiden yang tertinggi adalah di dalam bangsa Melayu. Hipotesis menyatakan bahawa terdapat peningkatan di dalam kekerapan sesetengah alel Kelas II (HLA-DRB1) dan terdapat peningkatan tahap HLA-DRB1 larut pesakit, sepertimana disokong oleh kajian terdahulu. Objektif kajian ini adalah untuk mengenal pasti HLA-DRB1 alel yang berhubung kait dengan ALL dalam kalangan pesakit Melayu di Malaysia, seterusnya menentukan jujukan DNA bagi alel risiko serta mengukur tahap HLA larut di dalam plasma pesakit. Penjenisan tisu HLA Kelas II telah dijalankan ke atas pesakit ALL; pre-B (n=42) dengan menggunakan teknik PCR-SSO (Tindak balas rantai polymerase-Jujukan khusus oligonukleotida) dan data genotip alel HLA tersebut dibandingkan dengan data dari penduduk berbangsa Melayu (n=1445). Tambahan lagi, tahap plasma HLA Kelas II larut (sHLA-DRB1) dalam pesakit (n=30) dan kumpulan kawalan (n=31) ditentukan dengan teknik Asai imunoerap terangkai enzim sandwic (ELISA). Jujukan DNA untuk HLA-DRB1 alel tertentu juga telah dijalankan di dalam exon 2 dengan melakukan pendekatan Penjenisan jujukan dasar (SBT). Pengagihan alel yang lebih tinggi pada HLA-DRB1 alel; HLA-DRB1*03 (nilai P=0.001, OR=2.92, 95% CI=1.47-5.80) dan DRB1*16 (nilai P=0.001, OR=2.76, 95 % CI=1.30-5.87) dilihat di kalangan pesakit yang menghidap ALL yang berbangsa Melayu. Sebaliknya, penurunan yang ketara dalam HLA-DRB1*07 (nilai P=0.032, OR=0.47, 95% CI=0.17-1.28) dan DRB1*12 (nilai P=0.008, OR=0.59, CI=0.33-1.03) telah dilihat dalam pesakit ALL, pre-B seterusnya mencadangkan potensi perlindungan terhadap penyakit. Analisis lanjut dari teknik SBT dapat menjelaskan resolusi alel risiko, iaitu

HLA-DRB1*03:01:01 dan HLA-DRB1*16:02:01. Polimorfisme dapat dilihat antara urutan exon 2 yang polimorfik dalam pesakit dan kumpulan kawalan bagi alel –DRB1*03 tetapi tidak dilihat pada –DRB1*16. Peningkatan dalam tahap larut HLA-DRB1 dalam plasma juga diperhatikan dalam pesakit ALL (0.260 ± 0.057 μg/mL) berbanding individu normal (0.051 ± 0.007 μg/mL) dengan nilai p=0.001. Keputusan yang diperolehi menyokong hipotesis awal yang mencadangkan bahawa gen HLA Kelas II antigen mudah terdedah kepada penyakit ini, dan berkemungkinan boleh dijadikan sebagai penanda bio untuk ALL. Kajian ini boleh menyumbang kepada etiologi dan patogenesis ALL dalam mengenal pasti HLA sebagai penanda risiko genetik yang berpotensi, dan juga sebagai salah satu penanda biologi kanser.

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I certify that a Thesis Examination Committee has met on 8 November 2013 to conduct the final examination of Norfarazieda binti Hassan on her thesis entitled "Association of Human leukocyte antigen with Precursor-B Acute lymphoblastic leukaemia (Pre-B ALL) in Malays" in accordance with Universities and University College Act 1971 and the Constitution of the University Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student should be awarded the Master of Science degree.

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DECLARATION

I declare that the thesis is my original work except for the quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

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LIST OF ABBREVIATIONS

-	Negative/minus
%	Percentage
±	Plus minus
+	Positive/plus
<	Less than
≤	Less than and equal to
=	Equal to
≥	More than and equal to
µg/mL	Microgram per litre
µL	Microlitre
^{0}C	Degree celsius
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
APCs	Antigen presenting cells
Av-HRP	Avidin-horseradish peroxidase
BMMCs	Bone marrow mononuclear cells
BMT	Bone marrow transplant
bp	Base pair(s)
BSA	Bovine serum albumin
CI	Confidence interval
CD	Cluster of differentiation
CLL	Chronic lymphoblastic leukaemia
CML	Chronic myeloid leukaemia
dH_2O	Distilled water

dNTPs	Deoxyribonucleotide(s)
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-Linked Immunosorbant Assay
FBS	Fetal bovine serum
g	Gram
GVHD	Graft-versus-host diseases
H_3PO_4	Phosphoric acid
HLA	Human leukocyte antigen
HSC	Haematopoietic stem cell
HTLV	Human T-lymphotropic virus
Ig	Immunoglobulin
kDa	Kilodalton
KIRs	Killer-cell immunoglobulin like receptors
L	Litre
LOH	Loss of heterozygosity
M	Molarity
Mb	Mega base pairs
MDS	Myelodysplastic syndromes
MFI	Median fluorescence intensity
mg	Milligram
mg/mL	Milligram per litre
$MgCl_2$	Magnesium chloride
MHC	Major histocompatibility complex
mL	Millilitre
mM	Milimolar

n	Number
Na_2EDTA	Disodium ethylenediaminetetraacetic acid
NaCl	Sodium chloride
$NaHCO_3$	Sodium bicarbonate
NCR	National Cancer Registry
ng/mL	Nanogram per litre
NGS	Next Generation Sequencing
NHL	Non-Hodgkin's lymphoma
NK	Natural killer
nm	Nanometer
OD	Optical density
OR	Odd ratio
PAX	Paired box protein
PBMCs	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCR-SSO	Polymerase chain reaction, Sequence-specific oligonucleotides
Pre-B ALL	Precursor-B Acute lymphoblastic leukaemia
Pre-T ALL	Precursor-T Acute lymphoblastic leukaemia
RBC	Red blood cell
rpm	Revolutions per minute
RPMI	Roswell Park Memorial Institute
SA-PE	Streptavidin-phycoerythrin
SBT	Sequence based typing
sHLA	Soluble Human leukocyte antigen

SNPs	Single nucleotide polymorphisms
SPSS	Statistical package for the Social sciences
Taq	*Thermus aquaticus*
TBE	Tris borate EDTA
TCR	T cell receptor
TdT	Terminal deoxynucleotidyl transferase
TE	Tris borate
TMB	3, 3', 5, 5' Tetramethylbenzidine
T1D	Type 1 Diabetes Mellitus
U/µL	Unit per microliter
V	Volt
WHO	World Health Organisation
X	Times
α	Alpha
β	Beta

CHAPTER 1

INTRODUCTION

The immune system utilises many complex processes to protect from infection by a variety of pathogens including bacteria, viruses and parasites. To function properly, these various components must be tolerant to self-antigens and evoke immunity against non-self antigens identified as invaders. The recognition of these components involves cell surface molecules. One of the most important molecules is the antigen-presentation by Major histocompatibility complex (MHC). These proteins are encoded by a large genomic region or gene family that is found in most vertebrates with the main role to present antigens to T cells (Delves and Roitt, 2000).

MHC molecules are polygenic being coded by multiple genes, in which a set is inherited with the paternal and maternal chromosomes. MHC molecules are highly polymorphic with multiple allelic forms for each MHC gene that exist in the population which makes it unlikely for two random individuals to express identical sets of genes (DeFranco, et al., 2007, p. 102-103).

The HLA antigens' most essential function is the induction and regulation of immune responses through T cell activation. Many studies have also reported the role of HLA antigens and its clinical relevance in the control of disease resistance and susceptibility by studying linkage disequilibrium. These associations are probable given the polymorphic nature of HLA antigens which determines its interactions with antigens in being poor or good presenter of certain virus or bacteria. Furthermore, close molecular similarity to the pathogen may cause the immune system to fail to recognise the pathogen as foreign and mount an immune response against it (Shankarkumar, 2004).

Such diseases that were studied include various types of cancers and tumours that are considered as complex and chronic disorders. As referred to by Ruiz-Cabello and Garrido (1998), it has been known for many years that tumours reveal altered expression of MHC class I and those changes in the HLA profiles were described before the role of MHC molecules in antigen presentation was discovered. This was thought to be a mechanism by which T-cell-resistant tumour-cell variants could be produced and selected with striking differences between classical (A, B, C) and non-classical (G, E) HLA class I molecules, in which the non-classical HLA family shows very little polymorphism compared to the classical HLA thus limiting its peptide–HLA–TCR interactions.

In describing the roles of HLA antigens in tumours, Bukur, et al., (2012) stated that the aberrant expression of HLA class I molecules can be caused by structural alterations or dysregulation of genes encoding the classical HLA class I antigens or components of the HLA class I antigen processing machinery (APM) in which could occur at the epigenetic, transcriptional or post-transcriptional level. These alterations provide tumour cells with different mechanisms to inactivate immune responses resulting in tumour growth and evasion from host immune surveillance. The example of the early HLA study on cancers has been described by Ford, et al. (1981) in which they had reported the abnormality of HLA antigens between the group of patients with lung cancers and normal to study the association of the malignancy with the

different histological types. In other types of cancer such as in cancer of blood and haematological diseases, some association and relationship between the disorders and HLA also have been demonstrated. There was evidence of a relationship between MHC and susceptibility to childhood leukaemia by analysis of the HLA-DR recessive genes in the family of children with Acute lymphoblastic leukaemia (ALL), as described by the early study (Von Fliedner, et al., 1983).

Leukaemia is defined as one of the haematological malignancies and a type of blood cancer that affects blood, bone marrow and lymph nodes and classified into acute or chronic and further subdivided into the cell types, myeloid or lymphoid. The patients that suffer from leukaemia will undergo damage of the bone marrow that replaces the normal bone marrow cells with higher numbers of immature white blood cells (Pui, 2006). However, there is no single cause known for all of the different types of leukaemia that are diagnosed and it is believed that different classification of leukaemia can be linked with different risk factors.

Like any other cancer and malignancy neoplasm, the concerns are mostly on genetic susceptibility and possible environmental factors, which can be natural or artificial, biological or chemical. Genetics is believed to play an important role for many diseases in order to develop the aetiology of disease by finding the relative risk. This can determine possibility of genetics relationship to a disease before studying specific gene related to that disease. The pathogenesis and epidemiologic studies of leukaemia in some populations strongly suggest that these diseases have an inherited basis and described an increase in homozygosis of HLA antigens that contribute as one of the risk factors. Thus, the HLA polymorphisms and different genetic make-up of different ethnic groups make it crucial to study the disease in specific ethnicity that eventually can help the researchers in understanding the mechanism of disease as well as to develop possible treatments. In this study, the emphasis is on the cases of pre-B Acute lymphoblastic leukaemia (pre-B ALL) in both adults and paediatrics (children) within the Malays. As referred to Oxford dictionaries, Malays refer to members of a people inhabiting Malaysia and Indonesia or a person of Malay descent.

It is hypothesised that there is an increase in frequencies of certain HLA-DRB1 alleles and also increase in soluble HLA-DRB1 in patients with pre-B ALL based on reports from the HLA and ALL association studies from other populations. Thus, the objectives of this study are (1) to identify alleles at the DRB1 locus of pre-B ALL patients (2) to sequence the HLA-DRB1 genes in pre-B ALL patients and normal individuals and (3) to determine soluble HLA-DRB1 levels in plasma of pre-B ALL patients and normal individuals.

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LIST OF PUBLICATIONS

Posters

1. Norfarazieda H, Dhaliwal JS, Hishamshah MI, Raudhawati O, Siti Zuleha I, Lee LJ and Maha A, Frequencies of HLA-DRB1 Alleles in Malay Patients with Acute Lymphoblastic Leukaemia, The Xth Malaysian National Haematology Scientific Meeting, 26th-28th April 2013, Georgetown Penang, Malaysia.

2. Siti Zuleha Idris, Norfarazieda Hassan, Lee Le Jie, Sabariah Md Noor, Raudhawati Osman, Hishamshah Mohd Ibrahim and Maha Abdullah, Chromosomal Translocation Screening of t(12:21) and t(8:21) in Acute Leukaemia Patients, The Xth Malaysian National Haematology Scientific Meeting, 26th-28th April 2013, Georgetown Penang, Malaysia.

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