ENHANCED IMMUNE CLEARANCE OF L-ASPARAGINASE AND ITS GENETIC PREDISPOSITION AMONG CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

TAN YAN QI

UNIVERSITI KEBANGSAAN MALAYSIA

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ENHANCED IMMUNE CLEARANCE OF L-ASPARAGINASE AND ITS GENETIC PREDISPOSITION AMONG CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKAEMIA

TAN YAN QI

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PENINGKATAN IMUN PENGHAPUSAN TERHADAP L-ASPARAGINASE DAN KECENDERUNGAN FAKTOR GENETIKNYA DALAM KALANGAN KANAK-KANAK YANG MENGHIDAPI LEUKEMIA LIMFOBLASTIK AKUT

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I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged.

26 August 2026

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P119905

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ABSTRACT

Pegylated L-asparaginase (peg-ASNase) is the cornerstone of childhood acute lymphoblastic leukaemia (ALL) chemotherapy. Its immunogenicity often prompts the production of antibodies, rendering the post-administration drug activity suboptimal in patients. Potential genetic variants may increase the risk of the drug's immune-mediated sequelae, with their allelic prevalences in the Southeast Asian patient population remaining obscure. The absence of validated commercial assays for quantifying serum peg-ASNase activity levels and anti-ASNase antibody titres further complicates the assessment of the drug effectiveness in patients. This study aimed (a) to develop and validate biochemical assays for measuring peg-ASNase activity levels and anti-ASNase IgG titres in human serum, (b) to determine the correlation between serum peg-ASNase activity and anti-ASNase IgG titres, (c) to investigate the association of seven genetic variants with serum anti-ASNase IgG titres, and (d) to determine the association between serum peg-ASNase activity and childhood ALL induction outcomes, mainly the marrow morphology status and minimal residual disease. A reversed-phase high-performance liquid chromatography assay with automated sample derivatisation was optimised and validated for measuring serum peg-ASNase activity (%CV range: 3.05%-4.16%; %error range: -3.03%-0.90%; LLOQ: 0.03 IU/mL). An indirect enzyme-linked immunosorbent assay was developed and validated to quantify human serum anti-ASNase IgG titres (%CV range: 6.54%-14.81%; %recovery range: 95.89%-100.77%; cut-off: ≥1.60 µg/mL). For genetic variants genotyping, conventional PCR and Sanger sequencing were performed. Statistical analysis was conducted using SPSS v29.0.1.0. Mean serum trough peg-ASNase activity levels were 0.11 IU/mL and 0.07 IU/mL for the newly diagnosed and relapsed patients, respectively, with 47.5% (29/61) and 20% (1/5) samples achieving ≥0.1 IU/mL in the respective cohorts after first drug dosing. There was an inverse correlation between serum peg-ASNase activity levels and anti-ASNase IgG titres (p=0.006). Patients with anti-ASNase IgG were found with lower serum peg-ASNase activity than those without antibody (p=0.002). *GRIA1* rs4958351 was inversely associated with post-administration anti-ASNase IgG titres, whereas *IL16* rs11556218 was associated with baseline antibody levels. Serum peg-ASNase activity levels were not associated with childhood ALL induction outcomes. This is the first local study on generic peg-ASNase, paving the way for future studies and improvements in drug administration for children using the developed laboratory assays.

ABSTRAK

Pegylated L-asparaginase (peg-ASNase) merupakan asas kepada kemoterapi untuk leukemia limfoblastik akut (ALL) kanak-kanak. Keupayaannya untuk memicu penghasilan antibodi berkemampuan menimbulkan aktiviti ubat yang tidak optimum dalam pesakit. Terdapat varian genetik yang berkecenderungan meningkatkan risiko kesan susulan imun terhadap peg-ASNase, dengan prevalensi alelik dalam kalangan pesakit yang berketurunan Asia Tenggara tetap tidak jelas. Ketidakwujudan analisis komersial yang mempunyai pengesahan untuk mengkuantifikasi tahap aktiviti peg-ASNase dan kepekatan antibodi anti-ASNase dalam sampel serum merumitkan lagi penilaian keberkesanan ubat tersebut dalam pesakit. Maka, kajian ini bertujuan untuk (a) menghasilkan dan mengesahkan ujian-ujian biokimia untuk mengukur tahap aktiviti peg-ASNase dan titer IgG anti-ASNase dalam sampel serum manusia, (b) menentukan kolerasi antara aktiviti peg-ASNase dan titer IgG anti-ASNase dalam serum, (c) menyiasat kaitan tujuh varian genetik dengan kepekatan IgG anti-ASNase serum, dan (d) menyelidik kaitan antara tahap aktiviti peg-ASNase dan hasil induksi ALL kanak-kanak, terutamanya status morfologi sumsum tulang dan penyakit sisa minimum. Ujian kromatografi cecair prestasi tinggi fasa terbalik dengan derivatisasi sampel automatik telah dioptimumkan dan disahkan untuk megukur aktiviti peg-ASNase serum (julat %CV: 3.05%-4.16%; julat %kesilapan: -3.03%-0.90%; LLOQ: 0.03 IU/mL). Ujian imunosorben berkaitan enzim yang tidak langsung telah dihasilkan dan disahkan untuk mengkuantifikasi titer IgG anti-ASNase serum manusia (julat %CV: 6.54%-14.81%; julat %pemulihan: 95.89%-100.77%; had: ≥1.60 μg/mL). Bagi penyiasatan genotip varian genetik, PCR konvensional dan penjujukan Sanger telah dijalankan. Analisis statistik telah dilaksanakan melalui SPSS v29.0.1.0. Purata tahap aktiviti palung peg-ASNase serum adalah 0.11 IU/mL dan 0.07 IU/mL masing-masing bagi pesakit yang baru didiagnosis dan yang mengalami relaps, dengan 47.5% (29/61) dan 20% (1/5) sampel mencapai ≥0.1 IU/mL dalam kohort masing-masing selepas dos ubat pertama. Tahap aktiviti peg-ASNase serum dan titer IgG anti-ASNase mempunyai korelasi songsang (p=0.006). Pesakit dengan IgG anti-ASNase positif mempunyai tahap aktiviti peg-ASNase serum yang lebih rendah berbanding pesakit tanpa antibody (p=0.002). *GRIA1* rs4958351 berkait secara songsang dengan titer IgG anti-ASNase selepas pemberian ubat, manakala *IL16* rs11556218 mempunyai kaitan dengan tahap antibodi asas. Tahap aktiviti peg-ASNase serum tidak dikaitkan dengan hasil induksi ALL kanak-kanak. Kajian ini merupakan kajian tempatan pertama mengenai peg-ASNase generik, berniat membuka jalan untuk kajian-kajian masa depan dan penambahbaikan pemberian ubat dalam kalangan kanak-kanak dengan ciptaan ujian makmal.

CONTENTS

LIST OF TABLES

LIST OF ILLUSTRATIONS

LIST OF ABBREVIATIONS

-Ve	negative
+Ve	positive
2N	2 Normal (= 1 Molar)
4PL	four-parameter logistic
5-SSA	5-sulfosalicylic acid
ACS	American Chemical Society
AHA	L-aspartic β-hydroxamate
a.k.a.	also known as
ALFA	Allele Frequency Aggregator
ALL	acute lymphoblastic leukaemia
ALL-BFM 2000	Acute Lymphoblastic Leukemia Berlin-Frankfurt-Münster Group 2000
ALL-REZ BFM 2002	Acute Lymphoblastic Leukemia-Relapse Study of the Berlin-Frankfurt-Münster Group 2002
AML	acute myeloid leukaemia
ANC	absolute neutrophil count
anti-ASNase	anti-asparaginase
ANZCHOG	Australian & New Zealand Children's Haematology/Oncology Group
Approx.	approximately
AR	analytical reagent
ASNase	L-asparaginase
ASNS	asparagine synthetase
ASNS	Asparagine Synthetase (Glutamine-Hydrolyzing)
ATP	adenosine triphosphate

AUC	area under the curve
BMA	bone marrow aspirate
BMI	body mass index
BSA	bovine serum albumin
CCG	Children's Cancer Group
CDC	U.S. Centers for Disease Control and Prevention
CDER	Center for Drug Evaluation and Research
CDR	Cytotoxic Drug Reconstitution
CI	confidence interval
CLL	chronic lymphocytic leukaemia
CML	chronic myeloid leukaemia
CNS	central nervous system
Co.	Company
Co. Ltd.	Company Limited
CSF	cerebrospinal fluid
dbSNP	database of single nucleotide polymorphism
DCFI	Dana-Farber Cancer Institute
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleotide triphosphates
E. coli	*Escherichia coli*
EcAI	*E. coli* type 1 L-asparaginase
EcAII	*E. coli* type 2 L-asparaginase
EDTA	ethylenediaminetetraacetic acid
e.g.	(*exempli gratia*): for example
ELISA	enzyme-linked immunosorbent assay

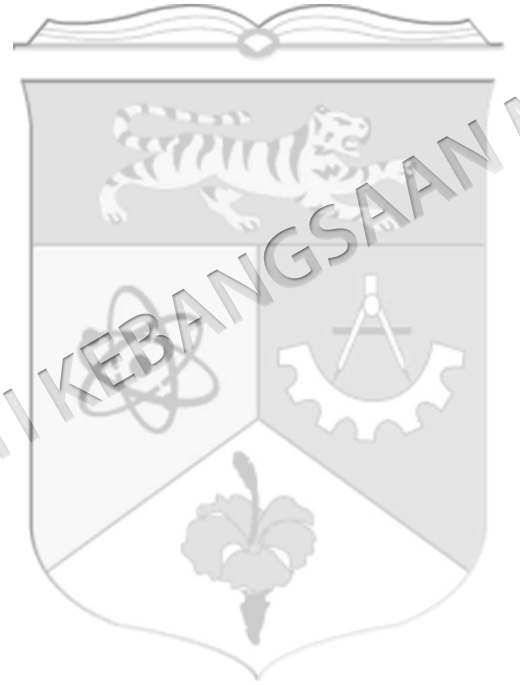
ErAII	*Erwinia* type 2 L-asparaginase
Erwinia	*Erwinia chrysanthemi*
etc.	(*et cetera*): and so on
EWAS	exome-wide association study
Fc	fragment crystallisable
FDA	United States Food and Drug Administration
GluA1	glutamate ionotropic receptor type subunit 1
GOT	glutamic oxaloacetic transaminase
GRIA1	Glutamate Ionotropic Receptor AMPA Type Subunit 1
GWAS	genome-wide association study
HCTM	Hospital Canselor Tuanku Muhriz
HIV	human immunodeficiency virus
HLA	Human Leukocyte Antigen
HPLC	high-performance liquid chromatography
HR	hazard ratio
HRP	horse radish peroxidase
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IDH	Italian, Dutch, Hungarian
i.e.	(*id est*): in other words
Ig	immunoglobulin
IL16	Interleukin 16
IL-16	interleukin-16
I.M.	intramuscular
Inc.	Incorporation

inj.	injection
INTERFANT-06	Infants Under One Year with Acute Lymphoblastic or Biphenotypic Leukemia
IQR	interquartile range
IS	internal standard
I.V.	intravenous
IVI	intravenous infusion
L-ASN	L-asparagine
L-ASP	L-aspartic acid
LDT	Laboratory Developed Test
L-GLU	L-glutamic acid
LLOQ	lower limit of quantification
LMIC	low- and middle-income countries
LOD	limit of detection
Ltd.	Limited
LU	luminescence units
MAAT	Medac Asparaginase Activity Test
MAF	minor allele frequency
MD	malic dehydrogenase
MHC	major histocompatibility complexes
MRD	minimal residual disease
MRM	multiple reaction monitoring
MYBBP1A	MYB-Binding Protein 1A
N/A	not available; not analysed
NCBI	National Center for Biotechnology Information

NCI	National Cancer Institute
NFAT	Nuclear Factor of Activated T Cells
NFATC2	Nuclear Factor of Activated T Cells 2
NGS	next generation sequencing
NHS	normal human serum
NMR	nuclear magnetic resonance
O.D.	optical density
OLS	ordinary least squares
OPA	*o*-phthaldialdehyde
OR	odds ratio
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PEG	monomethoxy-polyethylene-glycol
peg-ASNase	pegylated L-asparaginase
pH	potential of hydrogen
POG	Pediatric Oncology Group
PTFE	polytetrafluoroethylene
PTWG	Ponte di Legno Toxicity Working Group
QC	quality control
R&D	research and development
RNA	ribonucleic acid
ROC	receiver operator characteristic
RP-HPLC	reversed-phase high-performance liquid chromatography
S.D.	standard deviation
Sdn. Bhd.	(Sendirian Berhad): Private Limited

S.E.	standard error
SNP	single nucleotide polymorphism
SPEF2	Sperm Flagellar 2
SPR	surface plasmon resonance
SPSS	Statistical Package for the Social Sciences
TABTAR	Tunku Ampuan Besar Tuanku Aishah Rohani
TAE	Tris-acetate-EDTA
Taq	*Thermus aquaticus*
TDM	therapeutic drug monitoring
TMB	tetramethylbenzidine
Trizma®-HCl	Trizma® hydrochloride solution
UF	ultrafiltration
UHPLC	ultra-high-performance liquid chromatography
UHPLC-MS/MS	ultra-high-performance liquid chromatography coupled mass spectrometry
UK	United Kingdom
UKALL 2011	United Kingdom Acute Lymphoblastic Leukaemia and Lymphoma 2011
UKALL R3	United Kingdom Acute Lymphoblastic Leukaemia R3 Reinduction Regimen
UKM	Universiti Kebangsaan Malaysia
USA	United States of America
UV	ultraviolet
vs.	versus
WES	whole exome sequencing
WHO	World Health Organisation

WLS	weighted least squares
W/o	without
y/o	year-old



LIST OF UNITS

bp	base pairs
℃	degree Celsius
cm	centimetres
d	days
g	grams
Hz	Hertzs
h	hours
IU	international units
IU/mL	international units per millilitre
IU/m^2	international units per square meter
IU/m^2/dose	international units per square meter per dose
Kb	kilo bases
kDa	kilo Dalton
kg	kilograms
kg/m^2	kilograms per square metre
KU/mL	(similar as IU/mL)
L	litres
L/m^2/dose	litres per square meter per dose
M	molars
MΩ	megaohms
mg	milligrams
mg/m^2	milligrams per square meter
mg/mL	milligrams per millilitre
mg/m^2/d	milligrams per square meter per day

min	minutes
mL	millilitres
mL/min	millilitres per minute
mM	millimolar
mm	millimetres
µg/mL	micrograms per millilitre
µL	microlitres
µM	micromolar
µM/min	micromolar per minute
µm	micrometres
ng	nanograms
ng/mL	nanograms per millilitre
ng/µL	nanograms per microlitre
nM	nanomolar
nm	nanometres
s	seconds
V	voltages
x g	Relative Centrifugal Force

LIST OF SYMBOLS

%CV	coefficient of variation percentages
%error	error percentages
%recovery	recovery percentages
ρ	Spearman's rank correlation coefficient
C_{max}	maximum/peak plasma concentration
CL	clearance
N	sample size
n	sample size
R	Pearson correlation coefficient
R_s	resolution
r^2	coefficient of determination
$t_{1/2}$	half-life
T	retention time
T_a	annealing temperature
T_m	melting temperature
T_{max}	time to maximum plasma concentration
V_d	volume of distribution
W	width

LIST OF NOMENCLATURES

Asn	asparagine
Asp	aspartic acid
Ca	calcium
Ca^{2+}	calcium ions
Gln	glutamine
Glu	glutamic acid
H_2O	water
HCl	hydrochloric acid
K_2	dipotassium
K_2HPO_4	dipotassium phosphate
MeOH	methanol
Mg	magnesium
NAD^+	β-nicotinamide adenine dinucleotide
NADH	reduced β-nicotinamide adenine dinucleotide
NaOH	sodium hydroxide
NH_2	amino group
NH_3	ammonia
Lys	lysine

CHAPTER I

INTRODUCTION

1.1 BACKGROUND OF RESEARCH

Acute lymphoblastic leukaemia (ALL) is the most common type of childhood cancer which accounts for approximately 25% of the total malignancy cases diagnosed among children at <15 years old. In Malaysia, it is similar that leukaemia appears as the most prevalent childhood malignancy, with a prevalence of 34.3% in children aged 0 to 19 years old between 2012 and 2016 (Mohd Anis et al. 2019). Attributing to the availability of immunotherapy and targeted therapy, as well as with the recent advancement in the molecular risk stratification, the treatment outcomes for childhood ALL have improved tremendously over the decades, with an overall survival rate at >90%. Notably, the employment of L-asparaginase in the conventional chemotherapy regimens remains crucial for achieving an optimal treatment effect in the paediatric ALL patients.

L-asparaginase is used as a biopharmaceutical antineoplastic agent in the first-line chemotherapy regimens for childhood ALL. It is an enzyme that mainly catalyses the hydrolytic reaction of extracellular L-asparagine into L-aspartic acid and ammonia, depriving the leukaemia blast cells of the external sources of L-asparagine essential for their protein synthesis, in turn causing apoptosis and cessation of proliferation in these cancerous cells (Salzer et al. 2018). Since the 1970s, it was evidenced in clinical trials that patients who received L-asparaginase as the backbone of childhood ALL treatment protocols achieved better clinical outcomes compared to those without the enzymatic drug (Egler et al. 2016). The clinically available L-asparaginases are derived from two bacterial sources: *Escherichia coli* (*E. coli*) and *Erwinia chrysanthemi* (*Erwinia*). L-asparaginases of *E. coli* origin have been the cornerstone of childhood ALL induction

treatment, manufactured in either the native or pegylated form, with the latter covalently attached to the monomethoxy-polyethylene-glycol (PEG) polymers. Pegylated L-asparaginase has gradually replaced the conventional native *E. coli* formulation as a first-line agent in treating childhood ALL patients since the early 2000s in the developed countries, owing to its enhanced pharmacokinetics and reduced immunogenicity (Appel et al. 2008; Avramis & Panosyan 2005; Pieters et al. 2011).

As the L-asparaginase drugs are derived from bacterial sources, they are foreign to the human body and tend to be immunogenic which may stimulate the human immune system to generate anti-drug antibodies, provoking hypersensitivity reactions and a rapid drug clearance from the patients' bloodstreams (Shinnick et al. 2013). L-asparaginase hypersensitivity has two major categories: clinical hypersensitivity (a.k.a. allergic reaction) and subclinical hypersensitivity (a.k.a. silent inactivation). Patients with the clinical hypersensitivity towards L-asparaginases have manifestations such as fever, rashes, anaphylaxis, and so on, whereas patients with the silent drug inactivation will have an enhanced immune clearance of L-asparaginases in the absence of clinical symptoms (Schmiegelow et al. 2016; Schmiegelow et al. 2021). Among children with ALL, the prevalences of clinical hypersensitivities towards pegylated L-asparaginase ranged between 3% and 24% (Avramis et al. 2002; Raetz & Salzer 2010; Silverman 2001; Vrooman et al. 2010), while the prevalences of silent inactivation of the *E. coli* originated L-asparaginases ranged between 8% and 44% (Panosyan et al. 2004; Tong et al. 2014; Vrooman et al. 2013; Woo et al. 2000). Both the clinical and subclinical hypersensitivity of L-asparaginases would lead to a suboptimal enzyme activity level (<0.1 IU/mL) in the patients' bloodstreams post administration, more so occurring undetected during the events of silent drug inactivation under circumstances where the therapeutic drug monitoring (TDM) is not implemented for L-asparaginases. A subtherapeutic L-asparaginase activity level potentially results in an incomplete blood L-asparagine depletion, ultimately posing negative impacts on the overall childhood ALL treatment effectiveness (Van Der Sluis et al. 2016).

1.2 PROBLEM STATEMENT

On account of the prevalent clinical and subclinical hypersensitivity reactions towards L-asparaginases among children with ALL, which may swiftly attenuate the drug therapeutic efficacy with a suboptimal activity level post injection, the practice of TDM is crucial and increasingly advocated by clinical experts for paediatric patients who receive the drugs. While a number of bioassay protocols were developed and published by researchers for assessing L-asparaginase activity and anti-asparaginase antibodies in patients, there remains an absence of validated laboratory methods and commercial product or service for the clinical or research evaluation of such drugs in Malaysia. Existing gaps in knowledge and experience among the local analysts or technicians on the assessment of L-asparaginase activity and anti-asparaginase antibodies have stagnated the progression of TDM adoption as a standard of care in regional childhood ALL treatments. Particularly, it remains challenging to adopt or reproduce the published protocols in literature for determining anti-asparaginase antibodies, attributable to the highly volatile environmental and assay conditions per research setting basis.

The post-administration activity levels of L-asparaginases may be markedly reduced below the optimal therapeutic threshold of 0.1 IU/mL by the presence of anti-asparaginase IgG antibodies in patients' bloodstreams. Theoretically, the anti-asparaginase IgG level should inversely correlate with the L-asparaginase activity level, leading to an enhanced immune clearance of drug and causing a suboptimal drug activity with deficient antileukemic effect in patients. However, contradicting research findings on such hypothesis have rendered the theoretical correlation controversial and debatable. Despite L-asparaginase being a mainstay of childhood ALL treatment protocols for decades, the correlation between its drug activity level and the overall induction outcomes in patients remains understudied and poorly established in literature. The multifactorial and high interindividual variations in the post-administration L-asparaginase activity further complicate the elucidation of its drug effectiveness, more so when the generic drug formulation with unestablished bioequivalence data is applied clinically.

In addition, the frequencies of genetic risk alleles which associated with L-asparaginase hypersensitivity (*ASNS* rs3832526, *GRIA1* rs4958351, *HLA-DRB1* rs17885382, *IL16* rs11556218, *MYBBP1A* rs3809849, *NFATC2* rs6021191, *SPEF2* rs34708521) are not well-studied in the Southeast Asian populations. Research gaps in the statistical association between the genetic polymorphisms and the anti-asparaginase IgG synthesis in the local cohort of paediatric ALL patients are to be bridged, for gaining better insights into the pharmacogenetics of L-asparaginase hypersensitivities.

1.3 RESEARCH QUESTIONS

The research questions in this study were as follows:

1. How is the efficacy of the generic pegylated L-asparaginase utilised in the childhood ALL patients in this research setting?
2. Do the anti-asparaginase IgG titres inversely correlate with the pegylated L-asparaginase activity levels in serum samples of childhood ALL patients?
3. How is the distribution of the minor alleles of the seven proposed genetic variants in the childhood ALL patient population in Malaysia?
4. How do the target genetic variants associate with the serum anti-asparaginase IgG titres in children with ALL?
5. Do the serum pegylated L-asparaginase activity levels affect the childhood ALL induction outcomes?

1.4 OBJECTIVES OF STUDY

The general objectives of this research study were:

To investigate the therapeutic effectiveness of pegylated L-asparaginase post administration in the local childhood ALL patient cohort through measuring the drug activity levels and the anti-asparaginase IgG titres in patients' serum samples, using self-developed biochemical and immunological assays. Moreover, to determine the potential influences of the target genetic polymorphisms on the anti-asparaginase IgG

production in patients, as well as the potential effects of pegylated L-asparaginase activity on the childhood ALL induction outcomes.

Consequently, the specific objectives of this research study included:

1. To optimise and validate an automated reversed-phase high-performance liquid chromatography (RP-HPLC) assay method for measuring the pegylated L-asparaginase activity levels in serum samples of children with ALL during remission induction chemotherapy.
2. To develop and validate a quantitative indirect enzyme-linked immunosorbent assay (ELISA) method for measuring the anti-asparaginase IgG titres in serum samples of children with ALL during remission induction chemotherapy.
3. To determine the correlation between the pegylated L-asparaginase activity levels and anti-asparaginase IgG titres in children with ALL.
4. To determine the minor allele frequencies of the seven target genetic polymorphisms (*ASNS* rs3832526, *GRIA1* rs4958351, *HLA-DRB1* rs17885382, *IL16* rs11556218, *MYBBP1A* rs3809849, *NFATC2* rs6021191, *SPEF2* rs34708521) in the childhood ALL patient cohort in Malaysia.
5. To investigate the association between the target genetic polymorphisms and the serum anti-asparaginase IgG titres in children with ALL.
6. To determine the association between the serum pegylated L-asparaginase activity levels and the childhood ALL induction outcomes in terms of the bone marrow remission (BMA) and minimal residual disease (MRD) statuses.

1.5 SIGNIFICANCE OF RESEARCH

Development, optimisation and validation of the biochemical and immunoassay methods to enable the monitoring of serum drug activity levels and anti-drug antibody concentrations post administration of pegylated L-asparaginase in the childhood ALL patients in the local settings, as well as for the research applicability in the niche area.

By monitoring the serum pegylated L-asparaginase activity levels in patients' serum samples post drug administration, the efficacy of the employed generic formulation in induction could be assessed based on the overall trough drug activity and the proportion of patients achieving the consensus drug therapeutic threshold at ≥0.1 IU/mL. Besides, by revealing the presence or absence of anti-asparaginase IgG in the patients with suboptimal pegylated L-asparaginase activities, the actual events of enhanced immune clearance of the drug could be identified. In turn, clinicians may suggest either a switch of L-asparaginase preparations or a dosage adjustment for the prescribed formulation according to the laboratorial monitoring results, which may eventually enhance the treatment outcomes in childhood ALL patients.

Data on the genetic polymorphisms in this study may serve as a fundamental ground for further genetic exploration in the local patient population. It provides fundamental data for researchers to determine the likelihood of the paediatric ALL patients in Malaysia with Southeast Asian ancestral origins who possess the genetic risk alleles to be susceptible for L-asparaginase hypersensitivity or silent inactivation. Preliminary insights to the association between the target genetic polymorphisms and the serum anti-asparaginase IgG titres may potentiate future studies in a larger local childhood ALL patient cohort.

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