



UNIVERSITI PUTRA MALAYSIA

**SCREENING OF ALPHA-THALASSAEMIA 1 IN BETA- THALASSAEMIA
CARRIERS**

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THALASSAEMIA CARRIERS**

By

CHONG YI MIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Master of Science**

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For my Dad & Mom



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

SCREENING OF ALPHA-THALASSAEMIA 1 IN BETA-THALASSAEMIA CARRIERS

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August 2005

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Thalassaemia is an inherited blood disorder in which there is a reduction or absence in the synthesis of the globin chains of human Hb. Thalassaemia remains a public health problem in Malaysia, with many not knowing they carry the gene for thalassaemia. Individuals may be carriers of both α and β -thalassaemia. Concurrent α -thalassaemia 1 ($\alpha\alpha/--^{SEA}$) and β -thalassaemia (β^A/β^0) carriers are potential parents to offspring with Hb Bart's hydrops foetalis ($--^{SEA}/--^{SEA}$) and β -thalassaemia major (β^0/β^0). Hb Bart's hydrops foetalis results from homozygous state of α -thalassaemia 1 and β -thalassaemia major from homozygous β^0 .

This study determines the frequency of concurrent carriers of alpha and beta-thalassaemia. The information gathered from this study will aid government

agencies in policy-making, specifically on whether concurrent α -thalassaemia 1 identification needs to be done in any national screening programme for thalassaemia. Currently, most national screening programmes for thalassaemia including that in Malaysia concentrates on β -thalassaemia.

Blood samples were analyzed using conventional haematological methods. These include full blood counts/red cell indices followed by Hb analysis to quantify Hb subtypes by high performance liquid chromatography (HPLC). A thalassaemia carrier is presumptively identified by a cut-off value of $MCV < 80 \text{ fL}$ and $MCH < 27 \text{ pg}$. On HPLC, those with $HbA_2 > 4.0\%$ are identified as β -thalassaemia carriers. DNA was extracted from blood samples of the β -thalassaemia carriers and Gap-polymerase chain reaction (Gap-PCR) was done to identify the α -thalassaemia 1 molecular defect. The amplified product was run on 1.5% agarose gel by electrophoresis. The separated PCR product was then viewed under UV transillumination to identify the characteristic 570bp band for the α -thalassaemia 1 determinant.

A total of 231 β -thalassaemia samples were studied. Eight were found to have concurrently inherited the α -thalassaemia 1 ($--^{SEA}$) deletion, representing a carrier rate of 3.5%. The high carrier rate for α -thalassaemia 1 indicates the

need for the implementation of DNA analysis to complement thalassaemia diagnosis in a population screening programme. The relative risk of Chinese Malaysian to a non-Chinese being a concurrent carrier of α -thalassaemia 1 ($--^{SEA}$) and β -thalassaemia is 2.8 fold.

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

**SARINGAN ALPHA-THALASSAEMIA 1 DALAM PEMBAWA BETA-
THALASSAEMIA**

Oleh

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Thalassaemia ialah sejenis penyakit darah keturunan di mana sintesis rantai globin dalam hemoglobin manusia berkurangan atau langsung tidak hadir. Thalassaemia kekal sebagai masalah kesihatan awam di Malaysia, dengan ramai yang tidak tahu mereka sebenarnya pembawa gen thalassaemia. Seseorang individu boleh membawa kedua-dua gene α and β -thalassaemia. Pembawa serentak α -thalassaemia 1 ($\alpha\alpha/--^{SEA}$) dan β -thalassaemia (β^A/β^0) berpotensi untuk melahirkan anak yang mempunyai penyakit Hb Bart's hydrops foetalis ($--^{SEA}/--^{SEA}$) dan β -thalassaemia major (β^0/β^0). Hb Bart's hydrops foetalis disebabkan oleh keadaan homozygous α -thalassaemia 1 dan β -thalassaemia major oleh keadaan homozygous β^0 .

Kajian ini menentukan kadar pembawa serentak alpha dan beta-thalassaemia. Maklumat ini akan diberi kepada agensi kerajaan untuk menentukan sama ada identifikasi serentak α -thalassaemia 1 perlu dijalankan dalam program penyaringan awam thalassaemia. Buat masa ini, kebanyakan program penyaringan awam thalassaemia tertumpu pada β -thalassaemia, termasuklah yang dijalankan di Malaysia.

Sampel darah dianalisa dengan menggunakan kaedah hematologi konvensional, termasuklah pengiraan darah automasi/indices sel darah merah, diikuti dengan analisa hemoglobin oleh 'high performance liquid chromatography' (HPLC) untuk mengkuantifikasikan hemoglobin mengikut jenis. Pada mulanya, golongan yang mempunyai $MCV < 80 \text{ fL}$ dan $MCH < 27 \text{ pg}$ dianggap sebagai pembawa thalassaemia. Dengan HPLC, sampel yang mempunyai $HbA_2 > 4.0\%$ dikenali sebagai pembawa β -thalassaemia. DNA diekstrak dari sampel darah pembawa β -thalassaemia dan seterusnya 'Gap-polymerase chain reaction' (Gap-PCR) dijalankan untuk mengenalpasti kewujudan mutasi α -thalassaemia 1. Produk amplifikasi dianalisa atas gel agaros 1.5% dengan elektroforesis. Produk PCR yang dipisahkan dilihat dengan menggunakan cahaya UV untuk mengenalpasti saiz 570bp α -thalassaemia 1.

Sejumlah 231 sampel β -thalassaemia dikaji. Lapan dikenalpasti sebagai pembawa serentak yang mempunyai mutasi ($--^{SEA}$) α -thalassaemia 1. Ini mewakili kadar pembawa sebagai 3.5%. Kadar pembawa yang tinggi bagi α -thalassaemia 1 menunjukkan perlunya implimentasi analisa DNA bagi mengkomplementasikan diagnosis thalassaemia dalam program penyaringan awam. Peluang relatif seorang rakyat Malaysia berbangsa Cina dikenalpasti sebagai pembawa serentak α -thalassaemia 1 ($--^{SEA}$) dan β -thalassaemia berbanding dengan seorang rakyat Malaysia bukan Cina ialah 2.83 X.

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LIST OF ABBREVIATIONS

DCIP	Dichlorophenolindophenol
ddH₂O	Double-distilled water
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotriphosphate
EDTA	Ethylenediaminetetraacetic acid
FBC	Full blood count
Hb	haemoglobin
HLA	Human leukocyte antigen
HPLC	High performance liquid chromatography
HVR	Hypervariable region
IDA	Iron deficiency anaemia
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
OD	Optical density
OFT	Osmotic fragility test
PCR	Polymerase chain reaction

RBC	Red blood cells
RNA	Ribonucleic acid
SD	Standard deviation
SEA	Southeast Asia
UV	Ultraviolet
WHO	World Health Organization
bp	base pairs
kb(p)	kilo base pairs

CHAPTER 1

INTRODUCTION AND OBJECTIVES

1.1 Introduction

Thalassaemia is a disorder of haemoglobin (Hb) synthesis characterized by the absence or reduced synthesis of one or more of the globin chains, α , β , γ , δ , ϵ and ζ of human Hb. The two main types of thalassaemia that are clinically important are α and β -thalassaemia (Weatherall and Clegg, 2001).

Alpha-thalassaemia is the most common haemoglobin disorder in the world. Deletions of either one (α -thalassaemia 2) or both (α -thalassaemia 1) α -globin genes on chromosome 16 account for over 95% of α -thalassaemia cases (Higgs *et al.*, 1989).

In Southeast Asia, the form of mutation in α -thalassaemia 1 carriers is most commonly the SEA deletion ($--^{SEA}$). Alpha-thalassaemia 1 ($--^{SEA}$) carriers are at risk of having Hb Bart's hydrops foetalis offspring that usually dies *in utero* at the third trimester of pregnancy or shortly after birth

