UNIVERSITI PUTRA MALAYSIA

SCREENING OF ALPHA-THALASSAEMIA 1 IN BETA- THALASSAEMIA CARRIERS

CHONG YI MIN

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SCREENING OF ALPHA-THALASSAEMIA 1 IN BETA-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

August 2005
For my Dad & Mom
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 (αα/−SEAs) and β-thalassaemia (β^{+}/β^{0}) carriers are potential parents to offspring with Hb Bart’s hydrops foetalis (−SEAs/−SEAs) 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<80fL and MCH<27pg. On HPLC, those with HbA₂>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

CHONG YI MIN

Ogos 2005

Pengerusi: Profesor Elizabeth George, PhD

Fakulti: Perubatan dan Sains Kesihatan

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 (αα/-SEA) dan β-thalassaemia (β^+/β°) berpotensi untuk melahirkan anak yang mempunyai penyakit Hb Bart’s hydrops foetalis (−SEA/−SEA) dan β-thalassaemia major (β°/β°). Hb Bart’s hydrops foetalis disebabkan oleh keadaan homozygous α-thalassaemia 1 dan β-thalassaemia major oleh keadaan homozygous β°.
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 mengkuantifikasi hemoglobin mengikut jenis. Pada mulanya, golongan yang mempunyai MCV<80fL dan MCH<27pg dianggap sebagai pembawa thalassaemia. Dengan HPLC, sampel yang mempunyai HbA₂>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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I certify that an Examination Committee met on 19th August 2005 to conduct the final examination of Chong Yi Min on her Master of Science thesis entitled “Screening of Alpha-Thalassaemia 1 in Beta-Thalassaemia Carriers” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee are as follows:

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Date: 08 SEP 2005
DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

CHONG YI MIN

Date: 20/8/2005.
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<td>Dichlorophenolindophenol</td>
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<td>ddH₂O</td>
<td>Double-distilled water</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>dNTP</td>
<td>Deoxynucleotriphosphate</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>FBC</td>
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<td>Hypervariable region</td>
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<td>MCH</td>
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<td>Mean corpuscular volume</td>
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<td>kb(p)</td>
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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.