UNIVERSITI PUTRA MALAYSIA

HAEMATOLOGY, GENETICS AND MOLECULAR EPIDEMIOLOGY OF DELETIONAL ALPHA THALASSAEMIA IN MALAYSIA

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By

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Chairman: Professor Elizabeth George, M.D

Faculty: Medicine and Health Sciences

Alpha thalassaemia is the most common autosomal recessive single gene disorder in Southeast Asia, encountered in increasing numbers all over the world and heterogenous. Deletions of all four alpha thalassaemia genes (--/--) result in fetuses mostly die before or shortly after birth known as Hb Barts hydrops foetalis syndrome. It also caused serious maternal complications in pregnancies in which without medical care half were estimated to die**.** Deletions of 3 alpha thalassaemia genes (--/-α) is known as HbH disease with moderate anaemia, usually was similarly seen in the Malays and Chinese. In contrast, antenatal diagnosis for alpha thalassaemia reported Hb Barts hydrops foetalis (--/--) mostly in the Chinese. Thalassaemia studies among blood donors of 91.3% Malays found out that 30% were anaemic with all donors had a negative H-inclusion test, which is usually positive with double deletions (--/αα). Malaysia is a multiethnic country with mix marriages and 4.5% of Chinese-Malaysian is carriers of α^0

thalassaemia (--/). Therefore the purpose of this study was to determine the current deletional alpha thalassaemia status and deletional alpha thalassaemia burden in our population. Changes in deletional alpha thalassaemia gene frequency may change the deletional alpha thalassaemia burden. In this study haematology, genetics and molecular epidemiology of deletional alpha thalassaemia were looked at and carrier detection of common deletions was carried out. This will identify phenotypic characteristics, type of deletions and genotypes present, determine prevalence, estimate disease burden of deletional alpha thalassaemia and determine foetal genotype from pregnancies at risk of deletional Hb Barts hydrops foetalis in the country.

Standard haematology protocol, DNA studies and statistical analysis of qualitative and quantitative were applied. A cross sectional study was carried out on 405 samples. These were 238 EDTA blood from blood donors from Universiti Putra Malaysia, 15 DNA of 5 Hb Barts hydrops foetalis (--/--) and 10 alpha thalassaemia spouses (--/αα) from Universiti Malaya Medical Centre, 72 EDTA blood from HbH disease (--/-α) families from Institute for Medical Research and 80 DNA from blood donors from National Blood Bank. Blood count/blood film, HPLC, haemoglobin electrophoresis and multiplex PCR for detecting the 5 most common gene deletions were carried out. These are the common $--^{SEA}$ in Southeast Asia, the ethnic origins $--^{THAI}$ and $--^{FIL}$, the world common single deletions $-\alpha^{3.7}$ and $-\alpha^{4.2}$. Multiplex PCR using validated primers was developed using samples from families of HbH disease, detected the $--^{SEA}$, $--^{THAI}$, $--^{FIL}$ and $-\alpha^{3.7}$ giving 5 genotypes. This was compared with conventional method using samples from National Blood Bank and gave 100% accuracy, sensitivity and specificity

for $--^{SEA}$. $--^{FIL}$, $--^{THAI}$ and $-\alpha^{3.7}$ were also detected. In blood donors from Universiti Putra Malaysia, MCV < 80 fL was identified in 16.5% (with exclusion of Indians), deletional α–thalassaemia prevalence was 7.4% (3.5% double and 3.9% single deletions), equally distributed among Malays and Chinese. The most common double deletion detected was $--^{SEA}$ and $-\alpha^{3.7}$ for single followed by $-\alpha^{4.2}$. The prevalence was 5.3% in the Malays with the most common was $-\alpha^{3.7}$ (4.3%), 8.5% in the Chinese with 5.4% $--^{SEA}$ indicating an increase from 4.5% α^0 thalassaemia (--/) and 0.4% in others. All the $--^{SEA}$ were with MCV≤68 fL and MCH <22 pg. The projected number of pregnancies each year at risk of deletional Hb Barts hydrops foetalis syndrome and HbH disease are 30 and 120 in Malays, 250 and 150 in Chinese, 640 of both and on average 600 and 669 respectively. A typical α–thalassaemia phenotype was observed in HbH disease, and carriers of α^0 and α^+ thalassaemia. Real-time quantitative PCR (TaqMan®) with validated primers was optimized for $--^{SEA}$. Fetal genotype values of α^{SEA}-BR_N were 0.6 to 1.6 for ($--^{SEA}/--^{SEA}$), 0.2 to 0.5 for ($--^{SEA}/\alpha\alpha$) and 0 for ($\alpha\alpha/\alpha\alpha$) giving distinctly different values. The methods used were simple, reliable and useful in monitoring allele status. Identified carriers can be counseled and DNA from pregnancies at risk for (--/--) can be analysed. These will help in increasing awareness among carriers, bringing down deletional α–thalassaemia births and eventually lower the disease burden in Malaysia.

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

HEMATOLOGI, GENETIK DAN EPIDEMOLOGI MOLEKUL ALPHA THALASEMIA DELESI DI MALAYSIA

Oleh

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Alfa talasemia adalah kecacatan gen tunggal autosomal resesif paling lazim berlaku di Asia Tenggara, bilangannya semakin meningkat di seluruh dunia dan heterogenus. Delesi kesemua empat gen alfa talasemia (--/--) mengakibatkan fetus kebanyakkannya meninggal dunia sebelum atau sejurus selepas dilahirkan dan dikenali sebagai sindrom Hb Barts hydrops foetalis. Ia juga menyebabkan komplikasi maternal yang serius pada kandungan di mana tanpa penjagaan perubatan separuh dijangka meninggal dunia. Delesi 3 gen alfa talasemia (--/-α) dikenali sebagai penyakit HbH dengan anemia sederhana, selalunya dilihat samarata pada kedua-dua orang Melayu dan Cina. Walaubagaimanapun, diagnosis antenatal untuk penyakit alfa talasaemia melapurkan sindrom Hb Barts hydrops foetalis (--/--) kebanyakkannya pada orang Cina. Kajian talasemia dikalangan penderma darah yang 91.3% Melayu mendapati 30% adalah anemik dan kesemua penderma adalah negatif ujian H-inklusi, yang selalunya positif

dengan 2 delesi (--/αα). Malaysia adalah negara berbilang kaum dengan perkahwinan campur dan 4.5% daripada orang Cina-Malaysia adalah pembawa α^0 talasemia (--/). Oleh sebab itu tujuan kajian ini adalah untuk menentukan status terkini penyakit alfa talasemia delesi dan beban penyakit alfa talasemia delesi dalam populasi kita. Perubahan pada frekuensi gen penyakit alfa talasemia delesi boleh mengubah beban penyakit alfa talasemia delesi. Dalam kajian ini hematologi, genetik dan epidemologi molekul penyakit alfa talasemia delesi dilihat dan pengesanan pembawa delesi lazim dijalankan. Ini akan mengenalpasti sifat fenotip, jenis delesi dan genotip yang hadir, menentukan prevalen, menganggar beban penyakit alfa talasemia delesi dan menentukan genotip fetus pada kandungan berisiko sindrom Hb Barts hydrops fetalis delesi di negara ini.

Protokol hematologi piawai, kajian DNA dan analisis statistik kualitatif dan kuantitatif diaplikasikan. Kajian rentas dijalankan ke atas 405 sampel. Ini adalah 238 darah EDTA penderma darah dari Universiti Putra Malaysia, 15 DNA daripada 5 sindrom Hb Barts hydrops foetalis (--/--) dan 10 pasangan alpha talasaemia (--/αα) dari Pusat Perubatan Universiti Malaya, 72 darah EDTA ahli keluarga penyakit HbH (--/-α) dari Institut Penyelidikan Perubatan dan 80 DNA penderma darah dari Tabung Darah Negara. Kiraan darah/filem darah, HPLC, elektroforesis hemoglobin dan multiplex PCR untuk mengesan 5 delesi gen lazim dijalankan. In adalah delesi lazim $--^{SEA}$ di Asia Tenggara, mengikut asal etnik$--^{THAI}$, $--^{FIL}$, tunggal lazim di dunia $-\alpha^{3.7}$ dan $-\alpha^{4.2}$. PCR Multiplex menggunakan primer yang disahkan dibangunkan menguna sampel ahli keluarga pesakit

penyakit HbH, mengesan --^{SEA}, --^{THAI}, --^{FIL} dan -$\alpha^{3.7}$ dan memberi 5 genotip. Ini dibandingkan dengan kaedah konvensional menggunakan sampel dari Tabung Darah Negara dan memberi 100% betul, sensitif dan spesifik untuk --^{SEA}. --^{FIL}, --^{THAI} dan -$\alpha^{3.7}$ juga dikesan. Pada penderma darah Universiti Putra Malaysia, MCV<80fL dikenalpasti pada 16.5% (eksklusif orang India), prevalen α–talasemia delesi ialah 7.4% (3.5% --^{SEA} dan 3.9% delesi tunggal) dan ditaburkan samarata diantara orang Malayu dan Cina. --^{SEA} adalah delesi kembar dan -$\alpha^{3.7}$ adalah delesi tungal paling lazim dikesan diikuti -$\alpha^{4.2}$. Prevalen ialah 5.3% pada orang Melayu dengan -$\alpha^{3.7}$ (4.3%) paling lazim, 8.5% pada orang Cina dengan 5.4% --^{SEA} iaitu menunjukkan peningkatan daripada 4.5% α^0 talasemia (--/) dan 0.4% pada bangsa lain. Semua --^{SEA} mempunyai MCV$\leq$68 fL dan MCH <22 pg. Unjuran kandungan setiap tahun berisiko sindrom Hb Barts hydrops foetalis delesi dan penyakit Hb H delesi ialah 30 dan 120 pada orang Melayu, 250 dan 150 pada orang Cina, 640 pada keduanya dan secara purata 600 dan 669. Fenotip tipikal α–talasemia diperhatikan dari penyakit HbH, dan pembawa α^0 dan α^+ talasaemia. Kuantitatif PCR Real-time (TaqMan) menggunakan primer yang disahkan dioptima untuk (--^{SEA}). Nilai genotip fetus α^{SEA}-BR_N ialah 0.6 ke 1.6 untuk (--^{SEA}/--^{SEA}), 0.2 ke 0.5 untuk (--^{SEA}/αα) dan 0 untuk (αα/αα) memberi perbezaan nilai yang jelas. Kaedah-kaedah ujian yang digunakan adalah ringkas, boleh diharap dan berguna untuk monitor status alel. Pembawa yang dikenalpasti dapat diberi kaunseling dan DNA dari kandungan berisiko (--/--) alfa talasemia boleh dianalisis. Ini membantu meningkatkan kesedaran dikalangan pembawa, menurunkan kelahiran alfa talasaemia delesi dan seterusnya mengurangkan beban penyakit ini di Malaysia.

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

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DECLARATION

I declare that the thesis is my original work except for 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 at any other institution.

ALIZA BINTI MOHD YACOB

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