UNIVERSITI PUTRA MALAYSIA

ASSOCIATION OF ALPHA HAEMOGLOBIN STABILIZING PROTEIN IN HBE/BETA-THALASSAEMIA PATIENTS IN MALAYSIA

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ASSOCIATION OF ALPHA HAEMOGLOBIN STABILIZING PROTEIN IN HBE/BETA-THALASSAEMIA PATIENTS IN MALAYSIA

By

LIM WAI FENG

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

April 2011

♥ Specially dedicated to my lovely family members ♥

♥ my pleasure to go through life with you all ♥

♥ A reminder from them that we are so grateful to be in such a happy family ♥

♥ Thank you for being just the way you are ♥

♥ I love you all so much ♥

Abstract of 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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Chair: **Lai Mei I, PhD**

Faculty: **Medicine and Health Sciences**

β-thalassaemia is a condition caused by the quantitative reduction or absence of β-globin chain synthesis due to β-globin gene mutations. It leads to excessive unpaired α-globin chains precipitates which causes membrane damage and ineffective erythropoiesis. Haemoglobin E/β-thalassaemia patients have a remarkable variability in clinical severity due to globin chain imbalance and effects of other modifiers. Alpha haemoglobin stabilizing protein (AHSP) is an abundant erythroid protein that binds specifically to free α-globin chains to prevent its precipitation. Studies suggest that partial or full knockdown of AHSP may exacerbate β-thalassaemia phenotype in humans. This study was done to investigate the association between AHSP and HbE/β-thalassaemia patients in Malaysia. Peripheral blood samples from 105 patients were collected. Full blood count analysis and HPLC were carried out on the peripheral blood. Patients with

transfusions less than three months were excluded. In the end, 30 selected samples without underlying iron deficiency or co-inheritance of α-thalassaemia or extra α-genes were genotyped with ARMS PCR for common β-globin mutations and rare β-globin mutations were detected through sequencing. Common AHSP sequence variants were typed by tetra-primer ARMS PCR. TaqMan® quantitative RT-PCR was employed to correlate the AHSP expression to the severity and globin expression levels of HbE/β-thalassaemia. AHSP expression among 30 HbE/β-thalassaemia patients varied up to 4.50-log differences which was negatively correlated to MCH (p=0.009) and HbF (p=0.002), while positively correlated to alpha-globin expression level (p=0.003), beta-globin expression level (p=0.001) as well as excess alpha globins (p=0.004). The significant correlation between AHSP with MCH and HbF showed that AHSP increases when there are more unpaired α-globin chains. My study suggests that AHSP is a secondary compensatory mechanism to balance the excess α-globin chains in β-thalassaemia after the formation of HbF. This study further strengthens the theory that AHSP is a modifier for phenotypic severity in HbE/β-thalassaemia patients.

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

HUBUNGAN ANTARA ALPHA HAEMOGLOBIN STABILISING PROTEIN DAN PESAKIT HBE/BETA TALASEMIA DI MALAYSIA

Oleh

LIM WAI FENG

April 2011

Pengerusi: Lai Mei I, PhD

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β-Thalassemia adalah pengurangan kuantitatif atau tidak adanya sintesis rantai β-globin kerana mutasi gen β-globin yang mengarah berlebihan α-globin rantai yang tidak berpasangan ke presipitat yang menyebabkan kerosakan membran dan erythropoiesis tidak berkesan.HbE/β-thalassemia pesakit mempunyai variabilitas yang luar biasa dalam keparahan klinikal akibat ketidakseimbangan rantai globin dan kesan pengubah lain. Alpha Haemoglobin Stabilizing Protein (AHSP) ialah sebuah protein erythroid berlimpah yang mengikat secara khusus dengan rantai α-globin dan mengelakkan daripada presipitat. Kajian menunjukkan bahawa kehilangan secara penuh atau sebahagian AHSP boleh memburukkan fenotipe β-thalassemia pada manusia. Penyelidikan ini dijalankan untuk meneliti hubungan antara AHSP dan HbE/β-thalassemia pesakit di Malaysia. 105 sampel darah periferi dikumpulkan. Analisis hitung darah lengkap dan HPLC dilakukan pada darah periferi. Pesakit dengan pemindahan

darah kurang daripada tiga bulan tidak diproses. Pada akhirnya, 30 sampel dipilih tanpa defisiensi besi yang mendasari atau co-warisan daripada α-thalassemia atau tambahan α-gen dengan ARMS PCR untuk mutasi β-globin awam dan luar biasa β-globin mutasi dikesan melalui sekuensing. Variasi urutan umum AHSP yang ditaip oleh tetra-primer ARMS PCR. TaqMan® kuantitatif RT-PCR digunakan untuk mengkorelasikan ekspresi AHSP dengan tahap keparahan dan globin ekspresi pada HbE/β-talasemia. Ekspresi AHSP antara 30 HbE/β-talasemia pesakit berubah-ubah dengan perbezaan 4.50-log yang berkorelasi negatif dengan MCH (p=0.009) dan HbF (p=0.002), sedangkan berkorelasi positif dengan tahap ekspresi alpha globin (p=0.003), ekspresi beta globin (p=0.001) serta ekspresi alpha globin berlebihan (p=0.004). Signifi hubungan antara AHSP dengan MCH dan HbF menunjukkan bahawa AHSP meningkat sekiranya adanya kelebihan α-globin rantai yang tidak berpasangan. Pengajian saya menunjukkan bahawa AHSP adalah mekanisme pampasan sekunder untuk menyeimbangkan α-globin rantai yang berlebihan dalam β-thalassemia selepas pembentukan HbF. Penelitian ini semakin menguatkan teori bahawa AHSP adalah pengubah untuk keparahan fenotipik pada pesakit β-thalassemia.

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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.

LIM WAI FENG

Date: 12 April 2011

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