UNIVERSITI PUTRA MALAYSIA

HAEMATOLOGICAL PARAMETERS OF MALAY PATIENTS WITH COINHERITANCE OF SOUTHEAST ASIAN *OVALOCYTOSIS* AND *THALASSAEMIA HAEMOGLOBI-NOPATHY* TRAITS IN KLANG VALLEY, MALAYSIA

SHAHRZAD RIAHI

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By

SHAHRZAD RIAHI

Thesis submitted to the School of Graduate Studies of Universiti Putra Malaysia in the fulfilment of the requirements for the Degree of Master of Science

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DEDICATION

This thesis is dedicated to

My dearest Father and Mother

The understanding and encouragement they provided during all the these years

of the study

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

HAEMATOLOGICAL PARAMETERS OF MALAY PATIENTS WITH COINHERITANCE OF SOUTHEAST ASIAN *OVALOCYTOSIS* AND *THALASSAEMIA HAEMOGLOBINOPATHY* TRAITS IN KLANG VALLEY, MALAYSIA

By

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February 2013

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Southeast Asian Ovalocytosis (SAO) and thalassaemia-haemoglobinopathies are common inherited red blood cell (RBC) disorders in Southeast Asian countries. SAO, a RBC membrane defect is clinically asymptomatic. It is characterized by normal RBC parameters by in full blood count (FBC) with presences of oval stomatocytic RBCs in peripheral blood film. Thalassaemia-haemoglobinopathy, a globin defect of RBC is one of the major public health problems in Malaysia. Unlike SAO, thalassaemia-haemoglobinopathies is generally accompanied by hypochromic microcytic RBC parameters. Thalassaemia-haemoglobinopathies screening programme is mainly depends on evaluation of haematology (RBC) parameters. Therefore, its evaluations in the presence of those co-inheritance disorders are important to ensure the screening for thalassaemia-

haemoglobinopathies is optimum. **Objective:** To determine the prevalence of co-inheritance of SAO and thalassaemia-haemoglobinopathies in the Malay population and to evaluate the RBC parameters in those patients. **Method:** Prevalence of co-inheritance of SAO and thalassaemia-haemoglobinopathies was determined by recruiting 150 respondents among Malay volunteers' blood donors in UPM. Their blood sample were analysed for FBC, blood film, Hb analysis and serum ferritin. A total of 132 Malay patients with thalassaemia-haemoglobinopathies, SAO and co-inheritance of both were identified through samples for thalassaemia-haemoglobinopathies screening sent to laboratory haematology, Kuala Lumpur Hospital. These patients were consented and recruited to involve in this study. Their blood samples also were analysed for similar tests as blood donors. The DNA analysis was performed for respondents suspected of alpha thalassaemia (hypochromic microcytic RBC indices with normal serum ferritin and HPLC) and SAO (presence of stomatocytes in blood film). Single–tube multiplex PCR was used for confirmation of α-thalassaemia while PCR carried out to detect SAO. One-way ANOVA test using SPSS version 19 was used to analyse these data where *p*-value of < 0.05 was considered as statistically significant. **Results:** Prevalence of thalassaemia–haemoglobinopathies and SAO among blood donors like Hb E trait, β-thalassaemia trait, $\alpha^{3.7}$-thalassaemia, $\alpha^{4.2}$-thalassaemia, α^{SEA}-thalassaemia and SAO were 2.66%, 0.66%, 7.33%, 0.66% and 0.66% and 4.66% respectively. None of these donors was with co-inheritance of SAO and thalassaemia-haemoglobinopathies. All the participants in this study were divided into normal and anaemic groups according to their Hb level. The mean of RBC parameters in co-inheritance of SAO and Hb E such as Hb, MCV, MCH, MCHC, and RDW were 4.5 $x10^6$/μL, 10.33 g/dL, 66.33 fl, 22.5 pg, 33.96 g/dL,

18.3%, respectively. Among all the RBC indices, the mean MCV were significantly lower in co-inheritance cases as compared to respondents with only Hb E trait alone. The mean of RBC parameters in co-inheritance of SAO and β- thalassaemia trait such as RBC count, Hb, MCV, MCH, MCHC and RDW were of 3.98 x10^6/µL, 9.41 g/dL, 73 fl, 23.81 pg, 32.68 g/dL, 21.25%, respectively. Among these parameters, the RDW, MCV, and MCH were significantly higher and RBC count was significant lower in co-inheritance cases as compared to respondents with only β-thalassaemia trait alone. **Discussion:** This study showed respondents with co-inheritance of thalassaemia-haemoglobinpathies and SAO have lower MCV and MCH values as compared to reference standard in thalassaemia screening programme. The difference of the MCV value was significantly lower in co-inheritance of SAO and Hb E trait. The mean MCH in both groups of in co-inheritance was 22.5 pg and 23.8 pg, that has also lower value as compared to the references cut–off point, which were, being used for thalassaemia-haemoglobinopathies screening programme in Malaysia. **Conclusion:** Co-inheritance of SAO and thalassaemia-haemoglobinopathies does not have significant effect on cut-off values of RBC parameters which is, fundamental for thalassaemia-haemoglobinopathies screening.

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

PARAMETER HEMATOLOGI DIKALANGAN PESAKIT YANG MEMPUNYAI KEDUA-DUA MASALAH KETURUNAN SEL DARAH MERAH, SOUTHEAST ASIAN *OVALOCYTOSIS* DAN PEMBAWA PENYAKIT *TALASEMIA-HEMOGLOBINOPATI* DI LEMBAH KLANG, MALAYSIA

Oleh

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Southeast Asian Ovalocytosis (SAO) dan talasaemia-hemoglobinopati adalah penyakit keturunan melibatkan keabnormalan sel darah merah (SDM) yang biasa di kalangan penduduk di negara-negara Asia Tenggara. SAO, penyakit yang melibatkan keabnormalan pada membran SDM tidak mempunyai sebarang masalah klinikal. Pesakit SAO di cirikan oleh parameter SDM melalui ujian “full blood count” (FBC) yang normal , namun mempunyai SDM yang berbentuk stomatosit pada ujian calitan darah periferi. Talasaemia-hemoglobinopati yang diakibatkan oleh keabnormalan rantai globin pada SDM merupakan salah satu lagi masalah kesihatan awam yang ketara di Malaysia.Tidak seperti SAO, pesakit talasaemia-hemoglobinopati di cirikam oleh parameter SDM yang

secara kebiasaannya menunjuk SDM yang hipokromik mikrositik.Analisa parameter hematologi (SDM) merupakan ujian yang terpenting dalam ujian saringan talasemia-hemoglobinopati.Oleh yang demikian, kajian pada kesan parameter SDM dikalangan pesakit yang mewarisi kedua-dua masalah keturunan SDM ini secara bersama adalah amat penting bagi memastikan ujian saringan talasemia di Malaysia adalah ditahap yang optima.**Objektif:** Kajian ini bertujuan untuk mengetahui prevalens pesakit yang mengidapi kedua-dua penyakit keturunan SDM secara bersama dan seterusnya melihat kesan pada parameter SDM dikalangan mereka berbanding dengan peserta yang mengidap talassemia-hemoglobinopati sahaja. **Kaedah:** Prevalens pesakit yang mengidapi kedua-dua masalah keturunan SDM iaitu SAO dan talasemia-hemoglobinopati secara bersama ditentukan dikalangan 150 peserta yang direkrut dikalangan penderma darah berbangsa Melayu di Universiti Putra Malaysia (UPM). Sampel darah penderma dianalisa untuk ujian FBC, calitan darah periferi, ujian analisa hemoglobin dan tahap serum ferritin. Seramai 132 daripada pesakit berbangsa Melayu yang mengidap penyakit talasemia-hemoglobinopati, SAO dan kedua-duanya secara bersama telah dikenalpasti melalui ujian saringan talasemia yang di hantar ke Laboratori Hematologi, Hospital Kuala Lumpur. Pesakit yang memberi kebenaran dan bersetuju untuk turut serta dalam kajian ini telah direkrut. Sampel darah pesakit-pesakit ini telah dianalisa untuk ujian-ujian yang sama seperti yang dilakukan dikalangan penderma darah. Ujian DNA telah dilakukan pada semua peserta yang disuspek mengidapalfa-talasemia (parameter SDM yang hipockromik mikrositik tetapi mempunyai paras serum ferritin yang normal) dan SAO (calitan darah periferi menunjukkan terdapatnya sel stomatositik). Penyakit α-talasemia telah disahkan menggunakan kaedah “Single–tube multiplex PCR” manakala

SAO pula menggunakan kaedah PCR. Ujian ANOVAmenggunakan change to SPSS 19 Version telah digunakan untuk menganalisa data kajian dimana nilai-p yang <0.05 akan di anggap sebagai digit bermakna secara statistik. **Keputusan:** Prevalens penyakit talasemia-hemoglobinopati dan SAO dikalangan penderma darah di UPM untuk Hb E trait, β-thalassaemia trait, $\alpha^{3.7}$-thalassaemia, $\alpha^{4.2}$-thalassaemia, α^{SEA}-thalassaemia dan SAO adalah masing-masing seramai 2.66%, 0.66%, 7.33%, 0.66% , 0.66%. dan 4.66%. Tiada seorang pun dikalangan penderma darah yang dikenalpasti telah mengidap talasemia-hemoglobinopati dan SAO secara bersama.Peserta kajian ini telah dibahagi kepada kumpulan normal (tahap hemoglobin (Hb) yang normal) dan kumpulan anemia (tahap Hb yang kurang dari normal). Peserta yang dikenalpasti mempunyai masalah Hb E dan SAO secara bersama pula menunjukkan parameter SDM seperti RBC count, Hb, MCV, MCH, MCHC dan RDW adalah masing-masing 4.5 $x10^6$/μL, 10.33 g/dL, 66.33 fl, 22.5 pg, 33.96 g/dL, 18.3%. Purata MCV adalah lebih rendah dalam kumpulan masalah SAO dan talasemia-hemoglobinopati secara bersama berbanding dengan kumpulan pembawa Hb E sahaja. Peserta yang dikenalpasti mempunyai masalah β-thalassaemia trait dan SAO secara bersama pula menunjukkan parameter SDM seperti RBC count, Hb, MCV, MCH, MCHC dan RDW adalah masing-masing 3.98 $x10^6$/μL, 9.41 g/dL, 73 fl, 23.81 pg, 32.68 g/dL dan 21.25%. Kumpulan ini menunjukkan berbeza statistic yang bermakna pada nilai parameter RDW, MCV dan MCH dimana ianya lebih tinggi dan nilai RBC count yang lebih rendah berbanding dengan kumpulan pembawa β-thalassaemia sahaja.**Perbincangan:**Kajian ini menunjukkan responden yang mewarisi secara bersama talasemia haemoglobinpathies dan SAO mempunyai nilai MCV dan MCH yang lebih rendah berbanding nilai parameter tersebut yang digunakan untuk ru-

jukan standard dalam program saringan talasemia. Perbezaan nilai MCV adalah jauh lebih rendah dikalangan pesakit yang mewarisi penyakit talasemia-hemoglobinopati dan SAO secara bersama berbanding dengan kumpulan pembawa Hb E.Purata MCH dikalangan kedua-dua kumpulan yang mengidap talasemia-hemoglobinopati dan SAO secara bersamaadalah masing-masing 22,5 pg dan 23,8 pg, dimana nilai ini adalah lebih rendah berbanding dengan rujukan potong titik, yang digunakan untuk tujuan saringan talasemia-haemoglobinopathies di Malaysia**Kesimpulan:** Penyakit keturun-anSAO dan talasemia–hemoglobinopati yang diwarisi secara bersama tidak mempunyai sebarang kesan yang ketara pada nilai rujukan parameter SDM yang menjadi teras da-lam ujian saringan talasemia di Malaysia.

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I certify that a Thesis Examination Committee has met on 1/2/2013 to conduct the final examination of **Shahrzad Riahi** on his thesis entitled "**haematological parameters of malay patients with co-inheritance of Southeast asian ovalocytosis (SAO) and thalassaemia-haemoglobinopathies trait in Klang Valley "** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U. (A)] 15 March 1998. The Committee recommends that the Student be awarded the Master of Haematology.

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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 institutions

SHAHRZAD RIAHI

Date: 1 February 2013

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