UNIVERSITI PUTRA MALAYSIA

ASSOCIATION BETWEEN E-SELECTIN AND AMPD-1 GENE POLYMORPHISMS AND ESSENTIAL HYPERTENSION IN SELECTED MALAYSIAN SUBJECTS

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FPSK(m) 2013 21

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By

REZA NEMATI

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

April 2013

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DEDICATIONS

This thesis is dedicated to my beloved parents, particularly my father, Morteza Nemati, who supported and motivated me to have a higher education, to my lovely wife Maryam for their patient and extreme encouragement for me to accomplish my study and finally to my best friend Mohd Jokha Bin Yahya who helps me very much during my study.

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

ASSOCIATION BETWEEN *E-SELECTIN* AND *AMPD-1* GENE POLYMORPHISMS AND ESSENTIAL HYPERTENSION IN SELECTED MALAYSIAN SUBJECTS

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Chair: Prof. Patimah Ismail, PhD

Faculty: Medicine and Health Sciences

Essential hypertension (EH) is one the most common multifactorial disorders associated with significant risk for cardiovascular and renal comorbidity. Prevalence of hypertension is increasing annually in Malaysia. Studies indicate that the high prevalence of hypertension in this population is most common among males. Unfortunately, despite the high frequency of hypertension and its dread effects, few studies have been conducted on the Malaysian population. In contrast to the high rate of hypertension in Malaysia, hypertension prevalence is decreasing significantly in developed countries. A few studies have been carried out to explore primary hypertension in more detail among the Malaysian population. It is indicated that 30% to 50% of the

etiologic factors related to the development of essential hypertension are genetically-rooted. The aim of this current study was to determine the association of *E-selectin* and *Adenosine Monophosphate Deaminase1 (AMPD1)* genes polymorphism with essential hypertension among Malaysian subjects. The two genes were selected based on their function in the development of hypertension. As for the *E-selectin*, its functions are associated with pro-inflammatory effect, whereas for *AMPD1*, its influence on metabolism may be related in the etiology of hypertension.

Two hundred hypertensive and 200 normotensive individuals were recruited in this study, and their DNA were analyzed in order to determine the polymorphism of *E-selectin* and *AMPD1* genes. The PCR-RFLP method was used in this research. After extracting DNA using an available commercial DNA extraction kit, the DNA was incubated with the restriction enzyme to be cut into different fragments. Subsequently, post stain was carried out. To visualize DNA, the UV image capturing system was carried out to identify three forms of DNA pattern. There were significant associations observed for the selected gene polymorphisms and hypertension, namely, the S128R polymorphism of *E-selectin* (chi-squared, $p<0.05$) ; regarding *AMPD1,* for C34T, G468T and C143T (chi-squared, $p<0.05$). It is indicated that for the *E-selectin* S128R polymorphism, the R allele has a potent effect on essential hypertension (odds ratio 6.6, 95% CI 3.46-9.89); in addition, for the C34T, T allele carriers are 9.49 times more at risk of hypertension (odds ratio 9.49, 95% CI 5.6-16.02) . Furthermore, C143T subjects who are T carriers are 3.85 times more at risk of primary hypertension (odds ratio 3.85, 95% CI 1.86-6.70), while for G468T there was no difference observed with respect to both

alleles (odds ratio 1, 95% CI, 0.65-1.52). Also, there was a significant association observed between S128R polymorphism and increased level of SBP. Furthermore, in terms of SBP and DBP, there was a significant association observed among C34T genotypes. In this study, there was not significant relationship between smoking and gender based on different genotypes. In conclusion, this study shows the significant potential of *E-selectin* and *AMPD1* on the development of essential hypertension. These genes may be considered as a risk factor for subjects who are predisposed to hypertension. However, further studies which involve more samples and different populations need to be carried out.

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

PERKAITAN ANTRA *E-SELECTIN* DAN POLOMPFISME GEN *AMPD1* DENGAN HIPERTENSI DALAM SUBJEK MALAYSIA YANG DIPILIH

Oleh

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Hipertensi Esensial (EH) merupakan ketidaknormalan multifaktor yang mempunyai kaitan secara signifikan terhadap risiko menghidap penyakit kardiovaskular dan renal. Setiap tahun bilangan pesakit hipetensi meningkat di Malaysia. Banyak kajian menunjukkan bilangan penghidap hipertensi adalah lebih tinggi di kalangan lelaki dalam satu-satu populasi. Malangnya walaupun frekuensi penghidap hipertensi agak tinggi dengan gejala yang menakutkan, tidak banyak kajian yang telah dilaksanakan ke atas masyarakat Malaysia berbanding dengan negara-negara maju yang jumlah penghidapnya semakin berkurangan. Beberapa kajian yang terperinci berkenaan hypertensi primer telah dilakukan di Malaysia. Dari kajian ini 30% – 50% faktor etiologi hipertensi esensial berkait atau berasaskan genetik.

Matlamat Kajian ini adalah untuk menentukan kaitan gen *E-selectin* dan *Adenosine Monophosphate Deaminase 1* (*AMPD1*) dengan hipertensi esensial dikalangan subjek Malaysia. Kedua gen ini dipilih berdasarkan fungsinya dalam pembentukan hipertensi, *E-selectin* berkait dengan kesan pro-inflammatory manakala *AMPD1* pula berkait dengan metabolisme.

Dua ratus pesakit hipertensif dan individu normal telah dipilih dalam kajian ini dimana DNA individu ini telah dianalisis untuk menentukan polimorfisme gen yang dikaji. Dalam kajian ini metodologi yang digunakan ialah PCR-RFLP. DNA diekstrak dengan menggunakan kit ekstrak komersil dan dieram dengan enzim penghaduntuk dipotong menjadi fragmen dan diaplikasi pada *agarose* atau gel poliakrilamiddengan penanda. DNA dilihat dengan menggunakan sistem pencerap imej UV untuk mengenalpasti jenis fragmen DNA iaitu wild type*,* heterozigus dan homozigus. Terdapat perkaitan yang signifikan antara polimorfisme gen yang dikaji dengan risiko hipertensi. Polimorfisme pada S128R gen *E-selectin* (chi-squared, $p<0.05$); *AMPD1* pada C34T, G468T dan C143T (Chi-squared, $p<0.05$). Hal ini menunjukkan polimorfisme S128R pada *E-selectin* iaitu pada alel R mengakibatkan kesan yang poten dalam hipertesi esensial (odds ratio 6.6, 95% CI 3.46-9.89). Polimorfisme C34T pada gen *AMPD1* pembawa T mempunyai risiko mengidap ber potensi yang lebih tinggi iaitu 9.49 kali (odds ratio 9.49, 95% CI 5.6-16.02).Selanjutnya, bagi subjek C143T yang membawa polymorfisme T mempunyai 3.85 kali risiko untuk menghidapi hipertensi primer (*odd ratio* 3.85, 95% CI 1.86 – 6.70) manakala untuk G468T tiada perbezaan yang dapat diperhatikan untuk kedua alel tersebut (*odd ratio 1, 95% CI, 0.65 - 1.52*). Kesimpulannya, kajian ini membuktikan *E-selectin* dan *AMPD1*

mempunyai potensi yang signifikan dalam perkembayan penyalut hipertensi esensial. Gen-gen ini boleh dianggap sebagai faktor berisiko bagi individu yang terdedah pada penyakit hipertensi. Walau bagaimanapun kajian selanjut perlu dijalankan dengan bilangan sampel yang lebih banyak dan populasi yang berbeza.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my kind supervisor, Prof. Dr. Patimah Ismail who accepted me as a student, for her knowledge, generous guidance, encouragement, and her huge support that helps me to prepare this current thesis. I would also like to show my sincere acknowledgement to my advisors Associated Prof. Dr. Mansour Heidari and Dr. Reza Roozafsoon from Farabi Hospital, Tehran University of Medical and Health Sciences, Iran. Also, I would like to thank Dr. Amir Behnam Gharakhani from Razi Medical Lab, Robat Karim city, Iran. For their kindness and invaluable aids, as this research would not have been possible without their great supports. I am also indebted to my co-supervisor Dr. Suhaili Abu Bakar @ Jamaluddin, Zeinab Rabiee and Mahshid Shahrzad for their great guiding spirits, expert suggestions and constructive advices that had helped me in my study. I would like to thank my father, Morteza Nemati who supports me up to now. I would like to thank AbdoulHamid Arasteh and his wife Mahvash Asadi for their advice during my study.

I would like to thank all the nursing staff and the working doctors in Tuanku Jaafar Hospital for their assistance in recruiting the subjects for this study. I would like to thank all participants who participated in this research study.

I am grateful to my parents, my dear wife, and my sisters Nazanin and Nooshin for their love, support and extreme encouragement throughout the duration of my study.

I certify that a Thesis Examination Committee has met on 23 January 2013 to conduct the final examination of Reza Nemati on his thesis entitled "Association Between *E-selectin* and *AMPD1* Gene Polymorphism with Essential Hypertension in Selected Malaysian Subjects" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The committee recommends that the student be awarded the Master of Science.

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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.

REZA NEMATI

Date: 26 April 2013

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