UNIVERSITI PUTRA MALAYSIA

ASSOCIATION OF MATRIX METALLOPROTEINASE-1, 9, 12 AND TISSUE INHIBITOR OF METALLOPROTEINASE-1 GENE POLYMORPHISMS IN MALAY MALE ESSENTIAL HYPERTENSIVE SUBJECTS

FARIZEH AALAM GHOMI TABATABAEE

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By

FARIZEH AALAM GHOMI TABATABAEE

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirement for the Degree of Master of Science
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DEDICATION

Dedicated to My Beloved Parents

Abstract of thesis presented to the Senate of UniversitI Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

ASSOCIATION OF MATRIX METALLOPROTEINASE-1, 9, 12 AND TISSUE INHIBITOR OF METALLOPROTEINASE-1 GENE POLYMORPHISMS IN MALAY MALE ESSENTIAL HYPERTENSIVE SUBJECTS

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FARIZEH AALAM GHOMI TABATABAEE

September 2015

Chairman: Professor Patimah Ismail, PhD
Faculty: Medicine and Health Sciences

Genetic polymorphisms are the modified sequences of the DNA and they serve as molecular biomarkers for the detection of the individual at risk of developing the disease. Essential hypertension (EH) are majority of hypertensive cases and diagnosed where there is no clear evidence of medical condition predisposing to the high BP. There have been variety of the genetic studies in relation to hypertension and some of them showed association with occurrence of hypertension. Family of the matrix metalloproteinases (*MMP*) belong to the large family of the zinc-dependent endopeptidases that are involved in many physiological disorders ranging from cancer to cardiovascular disorders. Matrix metalloproteinases are implicated in degradation of the extracellular matrix (ECM) which is fundamental in many aspects, both physiologically and pathologically. These include: normal functioning of the cells from development to growth and proliferation, as well as pathological conditions such as cardiac remodeling and cancer development. Matrix metalloproteinases play important role in hypertensive vascular stiffness, remodeling and dysfunction. They may be involved in the excessive degradation of ECM components, vascular smooth muscle cells migration and proliferation and intima layer invasion by monocytes. Besides, ECM remodeling is largely determined by the balance of *MMPs* with respect to tissue inhibitor of metalloproteinases (*TIMP*). Several studies have been reported the imbalanced *MMP*:*TIMP-1* ratio in hypertensive subjects, indicating the depressed systematic degradation of collagenase in etiology of hypertension. The main objective of this study was to determine the candidate gene polymorphisms involved in ECM metabolism among Malaysian male subject with EH. Since, there have been variety of genetic association studies of *MMPs* and *TIMP*s conducted on different populations, but no study was done on Malaysian populations and in relation to hypertension. A total of 133 newly diagnosed EH subjects and 129 unrelated healthy individuals were requited under this study. The genomic DNA of these individuals were extracted from *buffy coat* and the plasma was separated for biochemical analysis. The genotyping of the polymorphisms were done by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. The PCR product and the restricted fragment product were run on agarose gel electrophoresis. All the statistical analysis were done by using Statistical Package for the Social Sciences (SPSS) version no. 21.0. The demographic characteristic of the subjects such as age, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (DBP), low density lipoprotein (LDL), triglyceride (TG) and cholesterol (Chol) were shown to be

differentially significant ($p < 0.05$) in case subjects when compared to the controls, high density lipoprotein (HDL) did not show any significance. The genotype and allelic distribution of *TIMP-1* 372 T/C polymorphism was highly significant in hypertensive subjects as compared to the controls ($p < 0.05$). Whilst, SNPs in position -1607 (1G/2G) in the *MMP-1* gene, position -1562 (C/T) and 279 (R/Q) of the *MMP-9* gene as well as site -82 (A/G) in the *MMP-12* gene did not differ significantly ($p > 0.05$) when compared to the controls. However, the data showed that the SNP in *TIMP-1* gene at site 372 (T/C) was associated with EH in Malay male hypertensive subjects. Hence, the allele and genotype of *TIMP-1* polymorphisms may be considered as a possible genetic biomarker and a risk factor for EH.

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

HUBUNG KAIT ANTARA METALOPROTEINASE MATRIKS-1, 9, 12 DAN DENGAN PERENCAT METALOPROTEINASE-1 POLIMORFISME GEN DI KALANGAN PESAKIT HIPERTENSI LELAKI MELAYU

Oleh

FARIZEH AALAM GHOMI TABATABAEE

September 2015

Pengerusi: Profesor Patimah Ismail, PhD
Fakulti: Perubatan dan Sains Kesihatan

Polimorfisme genetik adalah urutan DNA yang diubahsuai dan boleh digunakan sebagai penanda biologi molekul untuk mengesan individu yang berisiko mendapat sesuatu penyakit. Tekanan darah tinggi Essential adalah antara kes yang kebanyakan tidak mempunyai bukti yang sahih dari segi kaedah perubatan tentang keberkaitannya dengan tekanan darah yang tinggi dari golongan pesakit. Terdapat beberapa kajian telah dilakukan berkaitan dengan hubung kait antara genetik dengan terjadinya penyakit hipertensi, dan beberapa kajian ini dapat membuktikan kaitan di antara keduanya. Metaloproteinase matriks (*MMPs*) yang tergolong di dalam kumpulan *zinc-dependent endopeptidases* berkecenderungan kepada terjadinya gangguan fisiologi seperti kanser dan penyakit jantung. Metaloproteinase matriks terlibat dalam degradasi matriks ekstraselular (ECM) yang merupakan asas dalam banyak aspek, baik dari segi fisiologi dan patologi. Ini termasuk: fungsi normal sel-sel daripada pembentukan kepada pertumbuhan dan perkembangan, serta kondisi patologi seperti pembentukan semula jantung dan pembentukan kanser *MMPs* juga memainkan peranan penting dalam kekejangan vascular hipertensi, pembentukan semula dan disfungsi. Ia terlibat dalam degradasi komponen ECM yang berlebihan, migrasi vaskular licin sel-sel otot dan perkembangan, serta pencerobohan lapisan intima oleh monosit. Selain itu, sebahagian besar pembentukan semula ECM adalah ditentukan oleh baki *MMPs* berkenaan dengan tisu perencat metalloproteinases (*TIMP*). Beberapa kajian telah melaporkan ketidakseimbangan nisbah *MMP*:*TIMP-1* di kalangan pesakit hipertensi, membuktikan degradasi sistematik kolagenase dalam etiologi hipertensi. Objektif utama kajian ini dijalankan adalah untuk menentukan calon-calon gen polimorfisme yang terlibat dalam gen metabolisma matriks tambahan selular di kalangan pesakit hipertensi primer (EH) lelaki di Malaysia. Ini kerana, terdapat pelbagai kajian mengenai hubung kait genetik *MMPs* dan *TIMP*s dijalankan dalam populasi yang berbeza, tetapi masih tiada kajian yang dijalankan mengenai hubungannya dengan hipertensi di dalam populasi Malaysia. Sebanyak 133 orang yang baru didiagnos dengan EH dan 129 orang sihat yang tidak mempunyai hubungan dengan pesakit telah direkrut di bawah kajian ini. DNA genom individu-individu ini diekstrak daripada *buffy coat* manakala plasma diasingkan untuk analisis biokimia. Analisis genotip menggunakan kaedah *PCR* dan polimorfisme fragmen panjang restriksi (PCR-RFLP). Produk PCR dan produk RFLP dipisahkan dengan agaros gel elektroforesis. Semua analisis statistik menggunakan *Statistical Package for the Social Sciences* (SPSS) versi no. 21.0. Ciri-ciri demografi subjek seperti umur, indeks jisim badan, tekanan darah sistolik dan diastolik serta kadar

kolesterol lipoprotein berketumpatan rendah menunjukkan nilai perbezaan signifikan ($p < 0.05$) apabila dibandingkan antara kes dan kawalan. Manakala, kolesterol, trigliserida dan lipoprotein berketumpatan tinggi kolesterol tidak menunjukkan perbezaan yang signifikan. Pengagihan genotip dan alel 372 T/C polimorfisme daripada *TIMP-1* di kalangan subjek hipertensi mempunyai nilai signifikan yang tinggi berbanding kawalan ($p < 0.05$). Sementara itu, polimorfisme seperti *MMP-1*; -1607(1G/2G), *MMP-9*; -1562 (C/T), 279(R/Q) dan *MMP-12*; -82(A/G) di kalangan subjek hipertensi tidak mempunyai perbezaan signifikasi ($p > 0.05$) apabila dibandingkan dengan subjek kawalan. Walau bagaimanapun, data penyelidikan menunjukkan polimorfisme *TIMP-1*; 372(T/C) adalah mempunyai hubungan dengan EH dalam tekanan darah tinggi untuk subjek lelaki melayu.

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APPROVAL

I certify that a Thesis Examination Committee has met on 7 September 2015 to conduct the final examination of Farizeh Aalam Ghomi Tabatabaee on her thesis entitled **" ASSOCIATION OF MATRIX METALLOPROTEINASE-1, 9, 12 AND TISSUE INHIBITOR OF METALLOPROTEINASE-1 GENE POLYMORPHISMS IN MALAY MALE ESSENTIAL HYPERTENSIVE SUBJECTS"** in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

Member of the Thesis Examination Committee were as follows:

Name of Chairperson, PhD
Associate Professor Dr. Sabrina Sukardi
Faculty of Medicine
Universiti Putra Malaysia
(Chairman)

Name of Examiner , PhD
Dr . Abdah Md Akim
Faculty of Medicine
Universiti Putra Malaysia
(Internal Examiner)

Name of External Examiner, PhD
Title (e.g., Professor/Associate Professor/Ir; omit if irrelevant)
Name of Department and/or Faculty
Name of Organisation (University/Institute)
Country
(External Examiner)

ZULKARNAIN ZAINAL, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 5 November 2015

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

Patimah Ismail, PhD
Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

R.Vasudevan, PhD
Research Fellow
Institute of Gerontology
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT ,PhD
Professor and Dean
School of Graduate Studies
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LIST OF ABBREVIATIONS

BMI	Body Mass Index
BP	Blood Pressure
bp	base pair
CAD	Coronary Artery Disease
Chol	Cholesterol
CHD	Coronary Heart Disease
CVD	Cardiovascular disease
DBP	Diastolic Blood Pressure
ECM	Extracellular matrix
EH	Essential Hypertension
HDL	High Density Lipoprotein
HET	Heterozygous
HOM	Homozygous
LDL	Low Density Lipoprotein
mm Hg	millimeter of mercury
MMP-1	Matrix Metalloproteinase-1
MMP-12	Matrix Metalloproteinase- 12
MMP-9	Matrix Metalloproteinase- 9
MMPs	Matrix Metalloproteinases
PCR	Polymerase Chain Reaction
PCR-RFLP	PCR- Restriction Fragment Length Polymorphism
RE	Restriction Enzyme
SBP	Systolic Blood Pressure
SNP	Single Nucleotide Polymorphism
Ta	Annealing Temperature
TG	Triglyceride
TIMP-1	Tissue Inhibitor of Metalloproteinase-1
Tm	Melting Temperature
VLDL	Very Low Density Lipoprotein
VSMC	Vascular Smooth Muscle Cell
WT	Wild Type

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Hypertension is defined as the force exerted by the blood pressure (BP) to the walls of arteries as a result of heart beat, and when it is ≥ 140 millimeter of mercury (mm Hg) for systolic and ≥ 90 mm Hg for diastolic. While the normal BP of the individual is measured 120/80 mm Hg and below, the range above the normal and ≤139/89 mm Hg is considered as pre-hypertension (Clinical Practice Guidelines Management of Hypertension III, 2008). Hypertension can be classified as essential hypertension and secondary hypertension. Essential hypertension (EH) makes up to 95% of the cases of the hypertension and is diagnosed where there is no clear evidence of medical condition predisposing to the high BP (Chern and Chiang *et al.*, 2004). Whereas, the secondary hypertension making up the minority of the cases and are due to conditions such as Cushing syndrome, chronic renal failure or Conn's syndrome (Whelton, 1994).

There are number of factors contributing to hypertension such as life style, eating habits, genetic factors and presence of other medical complications leading to high BP. Less physical activity, high alcohol consumption, high salt intake, smoking and obesity are associated with elevated BP (Carretero and Oparil, 2000). While the role of environment had been known to impact the BP, the underlying genetic basis cannot be neglected. Essential hypertension is referred to as a complex genetic trait caused by multiple genes and these polygenic effects are controlled by gene-gene and gene-environment interactions (Chern and Chiang *et al.*, 2004).

Hypertension is characterized by increased vessel wall stress that leads to vascular remodeling and causing vascular resistance augmentation (Castro *et al.*, 2010). The vascular remodeling and augmented peripheral resistance in hypertension is marked by change in extracellular matrix (ECM) modification, accompanied by hypergenesis of vascular smooth muscle cells, leading to vascular stiffness as a result of thickened vessels (Intengan and Schiffrin, 2001, 2000).

Matrix metalloproteinases (*MMPs*) are a family of structurally related, zinc-dependent enzymes involved in excessive degradation of ECM components, vascular smooth muscle cell migration and proliferation (Visse and Nagase, 2003). Impaired *MMP* activity is involved in many clinical conditions affecting the cardiovascular system and plays an important role in hypertensive vascular remodeling and dysfunction including hypertension (Brionesa *et al.*, 2010; Humphrey, 2008; Raffetto and Khalil, 2008). Studies have shown that, the rise in *MMP* activity and expression is persistently

involved with vascular remodeling in hypertensive individuals and imbalance in *MMP*: Tissue Inhibitor of Metalloproteinas (*TIMP*) ratio particularly *TIMP-1*, may contribute to EH and hypertensive heart disease (Onal *et al.*, 2009; Ahmed *et al*., 2006; Yasmin *et al*., 2005; Laviades *et al.*, 1998).

The most stable variation of the genome occurs in the form of single nucleotide polymorphisms (SNPs) which make 90% of the common variations in the genome (Doris, 2002). Analyzing SNPs for the identification of loci associated with complex diseases are common in susceptibility to hypertension and other disorders. The SNPs association/candidate gene studies have revealed promising results in the genetic studies of complex diseases particularly in hypertension (Yagil and Yagil, 2009).

1.2 Problem Statement

Hypertension is a major risk factor for cardiovascular, cerebrovascular and renal diseases and it is considered as a polygenic disease and results from multiple gene-gene and gene-environment interaction (Deng, 2007; Chem and Chiag. 2004). Hypertension affects about 1 billion of worldwide population, and in Malaysia 4.8 million of individuals are hypertensive. The prevalence of the hypertension according to National Health and Morbidity Survey (NHMS) III issued on 2006 was more than 43%, included individuals aged ≥ 30 and the prevalence had shown to have increment of 30% from that reported 10 years earlier (NHMS III, 2006). However, the prevalence has increased only slightly from 32.2% in 2006 to the current 32.7%, an increase of about 0.5% (NHMS IV, 2011). In general, the prevalence of hypertension in the individuals aged ≥15 was shown to be 27.8% with higher prevalence in males (Rampal *et al.*, 2008).

Hypertension is characterized by increased vascular stress that leads to vascular resistant and remodeling (Humphrey, 2008). The hypertensive vascular remodeling is accompanied by reformation of ECM and vascular smooth muscle cells (VSMCs), bringing about vascular stiffness (Intengan and Schiffrin, 2000, 2001). While, the role of *MMP* and *TIMP* genes in normal ECM metabolism is well documented. Some studies have reported the imbalance in *MMP*/*TIMP* ratio in plasma level of hypertensive individuals, suggesting the impaired ECM metabolism (Androulakis *et al*., 2012; Castro *et al.*, 2010; Flammant *et al.*, 2007). Taking this into account, the present study was initiated to determine the association of genetic variations of *MMPs* and *TIMP-1* genes in Malaysian male hypertensive subjects. Since, there are lack of information in relation to ECM metabolism gene polymorphism in relation to hypertension in Malaysian population.

1.3 Significance of the Study

The candidate gene analysis would provide a better approach for identifying the genotype/phenotype and their probable correlations (Tabor *et al.*, 2002). The identification of the contributing genes for EH will allow the physicians to recognize the weak individuals. Also, classify the patients in subgroups with defining genetic and pathogenic mechanism which might enable the use of genotypes to identify more specific therapeutic and preventive measures.

Several studies have proposed the candidate genes for the susceptibility to hypertension in various populations (Agarwal *et al.*, 2005; Ruppert and Maisch, 2003). However, there are controversies in the results obtained from those studies and majority of these studies did not evaluate any possible interaction between different candidate genes. Hence, more studies using different population are needed in order to provide more information on genetic susceptibility of hypertension. Furthermore, to our knowledge there are lack of studies on the association of *MMPs* and *TIMP-1* gene polymorphisms with hypertension among Malaysians.

Genetic association analysis is to test whether an allele or genotype frequency differs between two groups and examines the statistical correlation between a person's genotype with his phenotype or disease. Most commonly the genetic association involves a study of the SNPs genotype frequency in a case-control study. According to this, association analysis can be used as a useful approach in studying the role of candidate genes in the development of multifactorial diseases (Lewis, 2002). The current study will enhance the management of hypertension among hypertensive subjects by choosing suitable drugs based on their genotypes.

1.4 Hypothesis

Gene polymorphisms of the *MMPs* and *TIMP-1* enzymes may be associated with the development of EH in Malay male hypertensive subjects.

1.5 Main objective

To determine if polymorphisms in *MMP-1, 9,12* as well as *TIMP-1*genes are associated with essential hypertension in Malay male subjects.

1.6 Specific objective(s)

1) To determine the genotypic and allelic frequency for -C1562T and R279Q polymorphisms of the *MMP-9* gene in Malay male subjects.

2) To determine the genotypic and allelic frequency for -1607 1G/2G polymorphism of the *MMP-1* gene in Malay male subjects.

3) To determine the genotypic and allelic frequency of –82A/G polymorphism of the *MMP-12* gene in Malay male subjects.

4) To determine the genotypic and allelic frequency of 372 T/C polymorphism of the *TIMP-1* gene in Malay male subjects.

5) To determine the relationship between genotypic, phenotypic and biochemical differences among Malay male subjects.

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