DNA METHYLATION IN ESSENTIAL HYPERTENSION IN YOUNG ADULTS IN EAST COAST MALAYSIA

BY

WAN FATEIN NABEILA WAN OMAR

A thesis submitted in fulfilment of the requirement for the degree of Doctor of Philosophy (Medical Sciences)

Kulliyyah of Medicine
International Islamic University Malaysia

JUNE 2020

ABSTRACT

Hypertension is emerging as the most prevalent risk factor of ischemic heart disease in young adults, but awareness is low in this age group. The prevalence of prehypertension in this population is also high, putting them at higher cardiovascular risk. The pathophysiology of essential hypertension has yet to be fully understood, and epigenetic modifications have been proposed to play some role. To date, very few epigenetic studies were done in young adults with prehypertension and hypertension. The aim of this study was to compare the level of DNA methylation in the promoter of implicated genes in young adults with normotensive blood pressure, prehypertension and hypertension. An observational cross-sectional study was conducted among 240 subjects age 18 to 45 years in Kuantan, Pahang, Malaysia. Eighty subjects were recruited for each blood pressure group; normotension, prehypertension, and hypertension as defined by the Ministry of Health Malaysia Clinical Practice Guidelines 4th edition. MethyLight analysis was performed to determine DNA methylation levels of *IL-6*, *ADD1* and *AGTR1* gene promoter in the blood. Differentially methylated genes in prehypertension and/or hypertension group were followed by gene expression study (n = 10 per group). There was no significant difference in *IL-6* methylation between hypertensive and normotensive. *IL-6* predicted prehypertension in males (p = 0.014), but not females. Hypertensive and prehypertensive males, and prehypertensive females, had lower *ADD1* methylation than their respective normotensive counterparts. After adjusting for other covariates, *ADD1* methylation predicted prehypertension and hypertension in males (p = 0.002 and p = 0.034 respectively). There was no significant difference in *AGTR1* methylation between the three groups in both sexes. There was no significant association between *IL-6* and *ADD1* methylation level and gene expression level. DNA methylation of *IL-6* and *ADD1* are independent predictors of prehypertension and/or hypertension in males hence has potential as an adjunct biomarker for risk stratification or disease progression. This is the pioneering study of *IL-6*, *ADD1* and *AGTR1* methylation in prehypertensive and hypertensive young adults. Further study to delineate potential mechanisms linking DNA methylation to disease development is warranted.

خلاصة البحث

يظهر فرط ضغط الدم عامل الخطر الأكثر إنتشارا لمرضي القلب الاقفاري لدي البالغين, لكن الوعي منخفض لهده الفئة العمرية. كما أن انتشار فرط ضغط الدم في هده الفئة العمرية من السكان مرتفع ايضا, مما يعرضهم لخطرأعلي لأمراض الأوعية الدموية والقلب. الفسيولوجيا المرضية لفرط ضغط الدم الأساسي لم يتم فهمه بالكامل, لّذلك تم إقتراح لتعديلات الجينيه لتلعب بعض الدور في هذا المرض. حتي الآن عدد قليل جدًا من الدرسات الجينيه لتي تم إجراؤها علي البالغين ما قبل فرط ضغط الدم وفرط ضغط الدم. وكان الهدف من هذه الدراسة هومقارنة مستوي ميثيل الحمض النووي في محفز الجينات لدي البالغين ذوي الضغط الطبيعي, وماقبل فرط ضغط الدم, وفرط ضغط الدم. أجريت دراسة مقطعية رصدية لعدد 240 شخصا تتراوح أعمارهم ما بين 18 الي 45 سنة في مدينة كونتان بولاية باهانج, ماليزيا. تم تحديد ثمانين شخصا لكل المجموعات من ضغط الدم الطبيعي, و ما قبل فرط ضغط الدم, وفرط ضغط الدم. وذلك طبقا للنحو المحدد من قبل وزارة الصحة الماليزية للممارسة التوجهية الطبعة الرابعة. تم إجراء تحليل ميثيل لايت (MethyLight) لتحديد مستوي ميثيل الحمض النووي من مروج الجينات (*ADD1*) و(*IL-6*) و(*AGTR1*) في الدم. الاختلافات في الميثيل الجيني لمجموعة ما قبل فرط ضغط الدم وفرط الضغط تبعت بدراسة تفصيلية للتعبير الجيني لعدد 10 أشخاص لكل مجموعة. لم يكن هناك فرق كبيرفي ميثيل (*IL-6*) بين مجموعة ضغط الدم الطبيعي و فرط ضغط الدم .*IL-6* تنبأ بما قبل فرط ضغط الدم للذكور ($p = 0.014$) ولكن ليس للإناث. الذكور المصابين بفرط ضغط الدم و ماقبل فرط ضغط الدم والأناث المصابين بما قبل فرط ضغط الدم كان لديهم اقل ميثيل للجين (*ADD1*) من الاشخاص ذو الضغط الدم الطبيعي لنفس الجنس. بعد تعديل المتغير ات المشتركة الاخرى, تنبأت مثيلة ADD1 قبل ارتفاع ضغط الدم وارتفا ع ضغط الدم لدي الذكور ($p = 0.002$, $p = 0.034$علي التوالي). لم يكن هناك فرق كبير في ميثيل (*AGTR1*) في الثلاثة مجموعات لكلا الجنسين. كذلك لم يكن هناك إرتباط كبير في ميثيل الجين (*IL-6*) ومستوي ميثيل (*ADD1*) ومستوي التعبير الجيني. ميثيل الحمض النووي ل *IL-6* و*ADD1* مؤشر تنبؤ مستقل لما قبل فرط ضغط الدم وفرط ضغط الدم عند الذكور. ومن ثم قد تكون مؤشر حيوي مساعد لتحديد مخاطر المرض وتطوره.هذه دراسه الرائده *AGTR1* و*ADD1* و*IL-6* لدي ا لشباب البالغين الذين يعانون من ارتقاع ضغط الدم وما قبل ارتفاع ضغط الدم. لذلك هناك مايبررالمزيد من الدرسات لتحديد الألية المحتملة التي تربط ميثيل الحمض النووي بنمو المرض.

APPROVAL PAGE

The thesis of Wan Fatein Nabeila Wan Omar has been approved by the following:

_____
Aszrin Abdullah
Supervisor

_____
Norlelawati A. Talib
Co-Supervisor

_____
Jamalludin Ab. Rahman
Co-Supervisor

_____
Azarisman Shah Mohd. Shah
Co-Supervisor

_____
Solachuddin Jauhari Arief Ichwan
Internal Examiner

_____
Abdul Rashid Abdul Rahman
External Examiner 1

_____
Muhammad Farid Johan
External Examiner 2

_____
Mohd Zulfaezal Che Azemin
Chairman

DECLARATION

I hereby declare that this thesis is the result of my own investigation, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Wan Fatein Nabeila Wan Omar

Signature……. Date ….....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

DECLARATION OF COPYRIGHT AND AFFIRMATION OF FAIR USE OF UNPUBLISHED RESEARCH

DNA METHYLATION IN ESSENTIAL HYPERTENSION IN YOUNG ADULTS IN EAST COAST MALAYSIA

I declare that the copyright holder of this thesis is International Islamic University Malaysia.

No part of this unpublished research may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording or otherwise without prior written permission of the copyright holder except as provided below

1. Any material contained in or derived from this unpublished research may be used by others in their writing with due acknowledgement.

2. IIUM or its library will have the right to make and transmit copies (print or electronic) for institutional and academic purposes.

3. The IIUM library will have the right to make, store in a retrieved system and supply copies of this unpublished research if requested by other universities and research libraries.

By signing this form, I acknowledged that I have read and understand the IIUM Intellectual Property Right and Commercialization policy.

Affirmed by Wan Fatein Nabeila Wan Omar

……..…….. ……..

Signature Date

ACKNOWLEDGEMENTS

Alhamdulillah, with His grace, this thesis is completing, and the author's journey is (finally) progressing onto the next chapter. Many believe that PhD is the beginning, rather than the end. So hello world, I am back!

It has been set as an indisputable rule, that only one person can author a thesis. Well, in case there is a change in the rule—though *very* unlikely—and I can choose a co-author, I would have put Ammar bin Yusop next to my name. My husband may not contribute to the intellectual and academic content of this thesis, but his endless support and assistance in many aspects of my non-academic life could not be emphasised too much.

Some people did thank their children whom without their presence could have expedited the whole process by at least half, duration-wise. I would not deny that sentiment wholly, but I choose to thank my three beautiful minions; Khaulah, Wam and Hilya, for being so loving and tolerating, although it was hard to comprehend my problem statements that I vented out. Thank you Umi, Mak and Abah, for keeping me (and this thesis) in your prayers.

Of course, my heartiest appreciation would definitely go to Dr Aszrin, whom I leisurely address as Kak Rin (with her consent of course). She goes way beyond being an academic advisor, a project supervisor or a postgraduate coordinator. Often, she becomes a mother—offering advice, logistic assistance and beyond, especially for someone who is relatively new to Kuantan. Sometimes, she is more like a sister, comforting and protecting her baby sister. But most of the times, she is a sahabah, a dear accompany whom I can pour my thoughts, feelings and views, minus the fear of judgement. For all these and more to come, thank you Kak Rin.

Dr Norlela is the chairperson of the supervisory committee. I am always at awe when she speaks. She is highly-knowledgeable, intuitive, very experienced, yet remained so humble. In my humble attempt at being at least one-tenth as good as her, I am (kind of) addicted to meet and learn from her but am guilty realizing that too much too long of an appointment could cost substantial delay in her reviewing her patients' results. I really appreciate Dr Lela's boundless commitment for academic consultation and delivering timely feedback in between the stack of documents to sign and H&E slides to review.

I wish to also write about Prof Jamal, Prof Azarisman, Kak Baiyah, Kak Shikin, Kak Huda, Dr Solah and every single soul who has showered me with unlimited assistance and unwavering support, but then it is gonna be too long. After all, I was advised that very few, *handpicked* people shall read the thesis; the student, supervisor(s) and examiners, that's about it. Thank you for reading (that is, *if* you do).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

A	Adenine
ACC	American College of Cardiology
ACE	Angiotensin converting enzyme gene
ACTB	Beta-actin gene
ADD1	α–Adducin gene
ADH	Antidiuretic hormone
ADRB	Adrenergic receptor gene
AGTR1	Angiotensin II Type 1 Receptor gene
AHA	American Heart Association
Alu	Sequence of Alu gene
Ang II	Angiotensin II
Anti-HPT	Anti-hypertensive medications
ANOVA	Analysis of variance
AT1aR	Angiotensin II Type 1 Receptor gene
B	Coefficient
baPWV	brachial–ankle pulse wave velocity
BMI	Body mass index
C	cytosine
CARDIA	Coronary Artery Risk Development in Young Adults Study
cDNA	Complementary DNA
CH_3	Methyl group
CI	Confidence interval
CO	Cardiac output
CPG	Clinical Practice Guidelines
CpG	Cytosine-phospodiesterase bond-guanidine
C_q	Quantitation cycle
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
DNMT	DNA methyl transferase
df	Degree of freedom
ECV	Effective circulating volume
EH	Essential hypertension
FBG	Fasting blood glucose
G	Guanidine
GAD	Generalised anxiety disorder
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase gene
GCK	Glucokinase gene
GRACE	Global Registry for Acute Coronary Effect
HbA1c	Glycosylated haemoglobin, type A1c
HDLC	High density lipoprotein cholesterol
Hpt	Newly-diagnosed hypertensive subjects
HR	Heart rate
hsCRP	High sensitivity C-reactive protein
HSD11β2	11-β-hydroxysteroid dehydrogenase 2 gene
ICAM–1	Intercellular adhesion molecule–1

IFN	Interferon gene
IL–1β	Interleukin–1β
IL-6	Interleukin-6 gene
IQR	Interquartile range
IREC	Institutional Research Ethics Committee
JNC	Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure
LINE	Long interspersed nuclear elements gene
LDLC	Low density lipoprotein cholesterol
MAP	Mean arterial pressure
MCP–1	Monocyte chemotactic protein–1
MDA	Malondialdehyde
MDD	Major depressive disorder
MLPD	Maternal low protein diet
MMF	Mycophenolate mofetil
MREC	Medical Research Ethics Committee
mRNA	Messenger ribonucleic acid
MSNA	Muscle sympathetic nerve activity
MTHFD	Methylenetetrahydrofolate dehydrogenase gene
n	Number
Na^+	Sodium
NCVD–ACS	National Cardiovascular Disease–Acute Coronary Syndrome Registry
NFKB	nuclear factor kappa B
NHMS	National Health and Morbidity Survey
NKCC	Sodium–potassium–chloride co–transporter gene
NMRR	National Medical Research Registry
Nt	Normotensive subjects
Obs/Exp CpG	Observed to expected CpG ratio
OR	Odd ratio
p	Significant level
PBL	Peripheral blood leukocytes
PCR	Polymerase chain reaction
PMR	Percentage methylation ratio
POMC	Proopiomelanocortin
PP	Pulse pressure
PR	Pulse rate
Pre	Prehypertensive subjects
R	Resistance in blood flow
r	Radius of blood vessel
r	Correlation coefficient
RAAS	Renin-angiotensin-aldosterone system
RM	Malaysian Ringgit
SBP	Systolic blood pressure
SCNN	Epithelial sodium channel gene
sd	Standard deviation
SE	Standard error
SHMT1	Serine hydroxymethyltransferase gene
SHR	Spontaneous hypertensive rats.
SNP	Single nucleotide polymorphism

SULF	Sulfatase gene
SV	Stroke volume
T	Thymine
TC	Total cholesterol
TC/HDL	Total cholesterol to high density lipoprotein ratio
TLR	Toll–like receptor
TNF–α	Tumor necrosis factor α
TPR	Total peripheral resistance
U	Uracil
USD	United States Dollar
VCAM–1	Vascular cell adhesion molecule–1
WHO	World Health Organisation
5mC	5-methylcytosine

CHAPTER ONE

INTRODUCTION

1.1 OVERVIEW OF STUDY

Cardiovascular diseases dictates the highest mortality and morbidity for non–communicable disease worldwide, accounting for approximately 17 millions death per annum, or one third of total death, and is projected to further rise in 2030 (World Health Organization, 2013). The main pathophysiology of cardiovascular disease is the development of atherosclerosis, which is associated with several risk factors including hypertension, obesity, smoking, dyslipidaemia, diabetes mellitus and family history of cardiovascular disease. Of all deaths due to cardiovascular disease , hypertension alone is responsible for 45 to 51 % of ischemic heart disease and stroke death (World Health Organization, 2013).

Hypertension was also the most prevalent cardiovascular disease risk factors among the acute coronary syndrome (ACS) patients in Malaysia according to the latest National Cardiovascular Disease–Acute Coronary Syndrome Registry (NCVD–ACS 2011–2013) (Wan Ahmad & Sim, 2015). This is contrasting the global data reported by the Global Registry for Acute Coronary Effect (GRACE) study in which smoking precedes the other cardiovascular disease risk factors (Global Registry for Acute Coronary Effect, 2007). It is also important to note that the mean age of ACS patients in Malaysia is approximately 6.5 years younger than other countries included in the GRACE study.

In 95 % of hypertension cases, there is no exact cause identified and therefore is termed as essential hypertension (Carretero & Oparil, 2000). National Morbidity

and Health Survey in 2011 indicated that as much as two–third of adults age 18 years and above have raised blood pressure, with almost half of the adult population have prehypertension—the pre-disease transition state between normotension to hypertension (Naidu et al., 2019). Furthermore, the awareness of hypertension among Malaysians is low, especially in younger age group of age 18 to 54 years (Institute for Public Health (IPH), 2015a). Additionally, prehypertension largely affects young adults and is associated with higher cardiovascular risk, especially in young adults (Egan & Stevens-Fabry, 2015; Elliott & Black, 2007). An earlier study in United States—the Framingham Heart Study—reported that up to 37 % of prehypertensive cases below 65 years, and up to 50 % above 65 years convert to hypertension in 4 years (Vasan et al., 2001). Meanwhile, a more recent local study indicated that the prehypertension–hypertension conversion rate was 69% in 10 years (Ching et al., 2012).

The exact cause of essential hypertension remains unknown although evidences have suggested that both genetic and environmental factors have roles in its pathophysiology (Carretero & Oparil, 2000; Kunes & Zicha, 2009). Nevertheless, several mechanisms were proposed to be involved, for example, inflammatory, abnormal sodium handling and the renin angiotensin aldosterone system (Montecucco et al., 2011; Orlov et al., 2014; Solak et al., 2016). Most studies into the pathophysiology of essential hypertension focused on genetic polymorphism, gene expression, and protein expression in these implicated pathways. Genetic–environmental interaction that underlies essential hypertension may be explained by the epigenetics phenomenon, in which alteration in the gene expression regulation occurs in response to environmental stimuli without changing the nucleotide sequence (Millis, 2011; Raftopoulos et al., 2015). One of the most understood epigenetic

mechanisms is deoxyribonucleotide acid (DNA) methylation. It is hypothesised that DNA methylation at the promoter region of a gene could alter the gene expression at the transcription level (D. H. K. Lim & Maher, 2010). Since modification of DNA methylation is implicated in many complex diseases from cardiovascular, metabolic, cancer and mental health diseases, it is proposed that DNA methylation could also affect the pathways involved in blood pressure regulation (Baccarelli, Wright, et al., 2010; D. H. K. Lim & Maher, 2010).

1.2 STATEMENT OF THE PROBLEM

DNA methylation serves the bridge linking between environmental factors and genetics onto phenotypes. Changes in DNA methylation has been identified to be involved in the pathophysiology of essential hypertension in adults; however its role in the pathogenesis of prehypertension and essential hypertension in young adults is not known. Furthermore, although some genes of interest have been studied, these pathways are yet to be fully explored. Based on the current literature, there were several unexplored areas that need to be addressed; 1) There is very limited literature on epigenetic studies in hypertensive young adults. 2) Available DNA methylation studies did not compare across three blood pressure status, i.e., normotension, prehypertension and hypertension, despite prehypertension as an established pre–disease position. 3) The different approaches and methods utilised to qualify or quantify DNA methylation results in varying, incomparable outcomes. 4) Furthermore, the studies were not extended to gene expression study; hence, the effect of differential DNA methylation on gene expression is largely unknown.