

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

**BY**

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## 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 4<sup>th</sup> 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 لهذه الفئة العمرية. كما أن انتشار فرط ضغط الدم في هذه الفئة العمرية من السكان مرتفع ايضا, مما يعرضهم لخطر أعلي لأعراض الأوعية الدموية والقلب. الفسيولوجيا المرضية لفرط ضغط الدم الأساسي لم يتم فهمه بالكامل, لذلك تم إقتراح لتعديلات الجينية لتلعب بعض الدور في هذا المرض. حتي الآن عدد قليل جدًا من الدراسات الجينية التي تم إجراؤها علي البالغين ما قبل فرط ضغط الدم وفرط ضغط الدم. وكان الهدف من هذه الدراسة هو مقارنة مستوي ميثيل الحمض النووي في محفز الجينات لدي البالغين ذوي الضغط الطبيعي, وما قبل 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 التوجيهية 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 كبير في 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 ما قبل فرط ضغط الدم والأناث المصابين بما قبل فرط ضغط الدم كان لديهم اقل ميثيل للجين (*ADD1*) من الاشخاص ذو الضغط الطبيعي لنفس الجنس. بعد تعديل المتغيرات المشتركة الأخرى, تنبأت مثيلة *ADD1* قبل ارتفاع ضغط الدم وارتفاع 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 الشباب البالغين الذين يعانون من ارتفاع ضغط الدم وما قبل ارتفاع ضغط الدم. لذلك هناك مايرالمزيد من الدراسات لتحديد الآلية المحتملة التي تربط ميثيل الحمض النووي بنمو المرض.

## **APPROVAL PAGE**

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## 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.

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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.

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## LIST OF ABBREVIATIONS

A	Adenine
ACC	American College of Cardiology
ACE	Angiotensin converting enzyme gene
<i>ACTB</i>	Beta-actin gene
<i>ADD1</i>	$\alpha$ -Adducin gene
ADH	Antidiuretic hormone
<i>ADRB</i>	Adrenergic receptor gene
<i>AGTR1</i>	Angiotensin II Type 1 Receptor gene
AHA	American Heart Association
<i>Alu</i>	Sequence of Alu gene
Ang II	Angiotensin II
Anti-HPT	Anti-hypertensive medications
ANOVA	Analysis of variance
<i>AT1aR</i>	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 <sub>3</sub>	Methyl group
CI	Confidence interval
CO	Cardiac output
CPG	Clinical Practice Guidelines
CpG	Cytosine-phosphodiesterase bond-guanidine
C <sub>q</sub>	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
<i>GAPDH</i>	Glyceraldehyde-3-phosphate dehydrogenase gene
<i>GCK</i>	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
<i>HSD11<math>\beta</math>2</i>	11- $\beta$ -hydroxysteroid dehydrogenase 2 gene
ICAM-1	Intercellular adhesion molecule-1

<i>IFN</i>	Interferon gene
IL-1 $\beta$	Interleukin-1 $\beta$
<i>IL-6</i>	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
<i>LINE</i>	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
<i>MTHFD</i>	Methylenetetrahydrofolate dehydrogenase gene
<i>n</i>	Number
Na <sup>+</sup>	Sodium
NCVD-ACS	National Cardiovascular Disease-Acute Coronary Syndrome Registry
NFKB	nuclear factor kappa B
NHMS	National Health and Morbidity Survey
<i>NKCC</i>	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
<i>p</i>	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
<i>r</i>	Radius of blood vessel
<i>r</i>	Correlation coefficient
RAAS	Renin-angiotensin-aldosterone system
RM	Malaysian Ringgit
SBP	Systolic blood pressure
<i>SCNN</i>	Epithelial sodium channel gene
sd	Standard deviation
SE	Standard error
<i>SHMT1</i>	Serine hydroxymethyltransferase gene
SHR	Spontaneous hypertensive rats.
SNP	Single nucleotide polymorphism

<i>SULF</i>	Sulfatase gene
SV	Stroke volume
T	Thymine
TC	Total cholesterol
TC/HDL	Total cholesterol to high density lipoprotein ratio
<i>TLR</i>	Toll-like receptor
TNF- $\alpha$	Tumor necrosis factor $\alpha$
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.