ASSESSMENT OF APOLIPOPROTEIN E AND PARAOXONASE 1 192Q/R GENE POLYMORPHISMS IN HYPERTENSION

BY

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ABSTRACT

Hypertension is a chronic cardiovascular disease that affects 43% of the Malaysian adult population. Hypertension is associated with significantly high morbidity and mortality rates. The exact cause of hypertension is still unknown but it is believed that hypertension occurs from complex interactions between multiple environmental and genetic factors. Although much is known about the environmental factors that predispose individuals to hypertension but the molecular mechanisms behind hypertension are still poorly understood. Between 30 and 60% of blood pressure variations are determined by genetic factors. A wide spectrum of genes has been studied for their possible role in the pathogenesis of hypertension, however very few genes have shown significant role in hypertension. Numerous recent studies on apoE and PON-1 proteins have shown that both proteins exhibit an anti atherosclerotic and antioxidant roles in the human body. Polymorphisms within the genes encoding these proteins have been linked with various pathologies including cardiovascular diseases, cognitive and infectious diseases. Apolipoprotein E (Apo E) is a plasma protein responsible for plasma clearance of triglyceride and cholesterol-rich lipoproteins. The ε2, ε3 and ε4 are the three common alleles of this gene. Paraoxonase-1 (PON-1) is an HDL associated esterase enzyme that inhibits LDL oxidation. One of the most important polymorphisms within this gene is the codon 192. This case control study consisted of 143 participants (seventy hypertensive and seventy three controls).The aim of this study was to assess the association of apolipoprotein E and PON-1 192 gene polymorphisms with hypertension and level of blood pressure. ApoE and PON-1 192 gene polymorphisms were analyzed by polymerase chain reaction-restriction fragment length polymorphism assay. The findings of this study suggest that polymorphisms in apoE and PON-1 192 were unlikely to confer genetic susceptibility for hypertension in our population samples.

خلاصة البحث

ارتفاع ضغط الدم هو مرض مزمن يؤثر على القلب والأوعية الدموية. حيث وجد ان 43 ٪ من السكان البالغين في ماليزيا يعانون من هذا المرض. ان ارتفاع ضغط الدم يرتبط مع ارتفاع معدلات الاعتلال والوفيات بشكل كبير. السبب الدقيق لارتفاع ضغط الدم لا يزال غير معروف ولكن يعتقد أن ارتفاع ضغط الدم يحدث من تفاعلات معقدة بين عدة عوامل بيئية ووراثية. وبالرغم من أنه يعرف الكثير عن العوامل البيئية التي تعرض الأفراد لارتفاع ضغط الدم لكن لا يزال فهم الآليات الجزيئية وراء ارتفاع ضغط الدم غير معروف. حيث انه قد اثبت ان ما بين 30- 60 ٪ من الاختلافات في ضغط الدم ناتجة عن طريق عوامل وراثية. وقد درس طيف واسع من الجينات لدورها المحتمل في التسبب في ارتفاع ضغط الدم ، ولكن عدد قليل جدا من الجينات أظهرت دورا هاما في ارتفاع ضغط الدم. وقد أظهرت الدراسات التي أجريت مؤخرا على بروتين ApoE وبروتين PON-1 أن كلا من البروتينين له دور في مكافحة تصلب الشرايين وايضا يعملان كمضادات الأكسدة في الجسم البشري. ان تعدد الأشكال داخل ترميز الجينات في هذه البروتينات قد ربط مع أمراض مختلفة بما في ذلك الأمراض القلبية الوعائية والإدراكية والأمراض المعدية. بروتين ال (ApoE) هو بروتين البلازما المسؤولة عن إزالة البلازما من الدهون الثلاثية والكولسترول والبروتينات الدهنية الغنية. و ε2، ε3 وε4 هي الأليلات الثلاثة الاكثر شيوعا لهذا الجين. اما بروتين ال (Paraoxonase – 1) او ما يعرف ب (PON-1) فهو انزيم مرتبط مع الكلسترول عالي الكثافه وله دور كبير في منع اكسدة الكولسترول الواطئ الكثافه. واحدة من أهم أشكال متعددة داخل هذا الجين هو كودون 192. تتألف هذه الدراسة من 143 مشتركا (سبعون يعانون من مرض ارتفاع ضغط الدم وثلاثه وسبعون من الناس الطبيعيين) ، والهدف من هذه الدراسة هو تقييم تعدد أشكال الجينات لكلا البروتينات مع ارتفاع ضغط الدم. وقد تم تحليل ذالك عن طريق فحص تفاعل التسلسل البوليميريزي للاشكال المتعددة المتقطعة الجزئية(RFLP). نتائج هذه الدراسة تشير إلى أن تعدد الأشكال في الجينات المشفرة لبروتين ApoE وبروتين PON1 من غير المرجح ان تمنح القابلية الوراثية لارتفاع ضغط الدم في عينتنا السكانية.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Master of Medical Sciences.

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DECLARATION

I hereby declare that this dissertation is the result of my own investigations, 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.

Wisam Nabeel Ibrahim

Signature …… Date ……..

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Signature Date

To My Parents

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TABLE OF CONTENTS

Abstract……..... ii
Abstract in Arabic..…… iii
Approval Page…..…… iv
Declaration……... v
Copyright Page……..… vi
Dedication…… vii
Acknowledgments……..… viii
List of Tables…….... xii
List of Figures…….. xiii
List of abbreviations…… xv

CHAPTER 1: GENERAL INTRODUCTION……...… 1

CHAPTER 2: LITERATURE REVIEW……..... 4
2.1 Hypertension…….. 4
2.1.1 Overview…… 4
2.1.2 Definition of blood pressure & high blood pressure ……..….. 4
2.1.3 Blood pressure measurement…… 6
2.1.4 Etiology & Risk factors of hypertension……..…… 6
2.1.4.1 Causes of hypertension…… 6
2.1.4.2 Risk factors for hypertension…… 7
2.1.5 Prevalence of hypertension…… 7
2.1.6 Morbidity & Mortality…….. 8
2.1.7 Physiological regulation of blood pressure…… 8
2.1.8 Pathophysiology of hypertension…….. 10
2.1.8.1 Reactive oxygen species …… 12
2.1.8.1.1 Signaling pathways & molecular targets of ROS…… 12
2.1.8.1.2 Vascular effects of ROS…….. 14
2.1.8.1.3 Mechanisms of oxidative damage…… 15
2.1.8.1.4 Antioxidants defense systems. …… 16
2.1.8.1.5 Linking oxidative stress with hypertension…… 17
2.1.8.2 Endothelial dysfunction in hypertension…… 18
2.1.8.2.1 Introduction…… 18
2.1.8.2.2 Pathophysiology of endothelial dysfunction…… 20
2.1.8.2.3 Endothelial dysfunction & oxidative excess…….. 21
2.1.8.2.4 Endothelial dysfunction & angiotensin и…… 22
2.1.8.2.5 Endothelial dysfunction & hypertension…… 22
2.1.8.3 Atherosclerosis…… 24
2.1.8.3.1 Introduction…… 24
2.1.8.3.2 Pathogenesis of atherosclerosis…… 24
2.1.9 Role of genetics in hypertension…… 29
2.2 Single nucleotide polymorphism…… 31

2.3 Apolipoprotein E..... 32
2.3.1 Historical background..... 32
2.3.2 ApoE gene polymorphism..... 33
2.3.3 Regulation of apoE gene expression..... 34
2.3.4 ApoE structure..... 34
2.3.5 ApoE synthesis..... 37
2.3.6 Physiological roles of apoE..... 38
2.3.7 ApoE & hypertension..... 44
2.4 Paraoxonase -1..... 44
2.4.1 Introduction..... 44
2.4.2 PON-1 gene polymorphisms..... 45
2.4.3 Structural bases of PON-1 activity polymorphism..... 46
2.4.4 Enzymatic characteristics & tissue distribution..... 47
2.4.5 Enzymatic activities of PON-1..... 48
2.4.6 Physiological roles of PON-1..... 49
2.4.7 Modulation of PON-1 function..... 52
2.4.8 PON-1 and hypertension..... 53
2.5 Hypothesis & objectives..... 55
2.5.1 Hypothesis of the study..... 55
2.5.2 Justification of the study..... 55
2.5.3 Objectives..... 55

CHAPTER 3: MATERIALS AND METHODS..... 56
3.1 Materials..... 56
3.1.1 Equipments..... 56
3.1.2 Reagents..... 56
3.2 Study design..... 56
3.3 Selection of subjects..... 58
3.3.1 Inclusion criteria..... 58
3.3.2 Exclusion criteria..... 58
3.4 Sample and data collection..... 59
3.4.1 Data collection..... 59
3.4.2 Blood pressure measurement..... 59
3.4.3 Blood sample collection..... 59
3.5 Genotype determination..... 60
3.5.1 Isolation of genomic DNA..... 61
3.5.2 Spectrophotometry..... 63
3.5.3 Agarose Gel Electrophoresis of Genomic DNA 63
3.5.4 Genotype analysis..... 65
3.5.4.1 ApoE Polymerase Chain Reaction..... 67
3.5.4.2 ApoE restriction enzyme digestion..... 69
3.5.4.3 PON-1 192Q/R Polymerase Chain Reaction 72
3.5.4.4 PON-1 192Q/R restriction enzyme digestion..... 74
3.6 Statistical analysis..... 75

CHAPTER 4: RESULTS 76
4.1 Study population..... 76
4.2 Isolation of genomic DNA..... 78
4.3 Genotype analysis..... 78

4.3.1 ApoE PCR…… 78
4.3.2 ApoE restriction enzyme digestion…….. 79
4.3.3 PON-1 192Q/R PCR…… 81
4.3.4 PON-1 192Q/R restriction enzyme digestion…… 81
4.4 Genotype and allele frequencies……..……. 82

CHAPTER 5: DISCUSSION …… 86

CHAPTER 6: CONCLUSION…… 92

BIBLIOGRAPHY …… 93

APPENDIX I…… 111

APPENDIX II…… 116

APPENDIX III…… 122

LIST OF TABLES

Table No.		Page No.
2.1	Classification of hypertension	5
3.1	Primers used in genotyping assay	68
3.2	PCR program used for apoE amplification	68
3.3	Restriction enzymes with their recognition sequence	70
3.4	PCR program used for the amplification of PON-1 192Q/R	73
4.1	Demographic data	77
4.2	Age & blood pressure in hypertensives & controls	77
4.3	Distribution of genotypes & alleles frequencies in hypertensive patients and controls	83
4.4	Blood pressure distribution in apoE and PON-1 192 genotypes	84
4.5	Distribution of genotypes frequencies in males and females	85
A.1	List of Study population	116

LIST OF FIGURES

Figure No.		Page No.
2.1	Pressure changes in the aorta (red line) and left ventricle (blue line) during systole and diastole	4
2.2	Pathophysiology of hypertension	10
2.3	Redox dependant signalling pathways in vascular cells	13
2.4	Vascular effects of reactive oxygen species	14
2.5	Endothelial physiological roles	18
2.6	Regulatory functions of the endothelium	19
2.7	A model for early steps in the development of the atherosclerotic lesion	25
2.8	Isoelectric focusing showing the three homozygous apoE phenotypes	32
2.9	ApoE structure	35
2.10	Three dimensional structure of apoE highlighting isoforms differences	36
2.11	Role of apoE in lipid metabolism	39
2.12	Physiological roles of macrophages apoE	43
2.13	HDL dependant PON-1 structure	47
2.14	Structure of PON-1	48
2.15	Anti atherogenic functions of PON-1	50
2.16	PON-1 anti atherogenic effect on macrophages	51
3.1	Flow chart of the experimental design	57
3.2	Flow chart of genotyping	60
3.3	DNA & protein sequence of apoE target region	71

3.4	Schematic presentation of apoE genotypes on agarose gel electrophoresis	71
3.5	DNA & protein sequence of PON-1 192Q/R target region	73
3.6	Schematic presentation of PON-1 192Q/R SNP enzymatic digestion and allele determination on gel electrophoresis	74
4.1	Agarose gel electrophoresis of isolated genomic DNA	78
4.2	Agarose gel electrophoresis of amplicon products of apoE target region with the expected DNA product of 244 bp	79
4.3	Agarose gel electrophoresis of the digested products of apoE amplicons	80
4.4	Agarose gel electrophoresis of amplicon products of PON-1 192Q/R region with expected DNA products of 99 bp	81
4.5	Agarose gel electrophoresis of digested products of PON-1 192Q/R amplicons	82

LIST OF ABBREVIATIONS

A	Adenine
ABCA1	ATP binding cassette transporter A1
ABPM	Ambulatory blood pressure monitoring
AlWl	Acinetobacter lwoffii endonuclease enzyme
Ang. II	Angiotensin II
ANP	Atrial natriuretic peptide
AP-1	Activator protein -1
ApoE	Apolipoprotein E
Arg.	Arginine
BH_4	Tetrahydrobiopterin
BMI	Body mass index
BP	Blood pressure
bp	Base pair
C	Cytosine
C.O.	Cardiac output
cAMP	Adenosine 3' – 5' cyclic monophosphate.
Cys.	Cystein
DH	Dipping hypertension
DMSO	Dimethyl sulfoxide.
DNA	Deoxyribo Nucleic Acid.
dNTPs	Deoxynucleoside triphosphates.
ECM	Extracellular matrix proteins
EDTA	Ethylenediaminetetraacetic acid.
eNOS	Endothelial nitric oxide synthase
ET-1	Endothelin-1
FFA	Free fatty acids
G	Guanine
GSH	Glutathione
HDL	High density lipoprotein.
Hhal	Haemophilus haemolyticus endonuclease enzyme
HSPG	Heparan sulphate proteoglycans
HWE	Hardy–Weinberg equilibrium.
ICAM-1	Intracellular adhesion molecule- 1
IDL	Intermediate density lipoprotein
IL-6	Interleukin-6.
LCAT	Lecithin-cholesterol acyltransferase
LDL	Low density lipoprotein.
LPC	Lysophosphatidylcholine

LRP	LDL receptor–related protein
MAPK	Mitogen-activated kinases
MMPs	Matrix metalloproteinases
NADPH	Nicotinamide adenine dinucleotide phosphate
NCBI	National centre for biotechnology information
NDH	Non dipping hypertension
NFkB	Nuclear Factor-KappaB
NO	Nitric oxide
PCR	Polymerase chain reaction.
PKA	Protein kinase.
PON-1	Paraoxonase-1
PR	Peripheral resistance
PTK	Protein tyrosine kinases
PTP	Protein tyrosine phosphatases
Q/R	Glutamine /Arginine
RAS	Rennin angiotensin system.
RE	Restriction endonuclease.
RFLP	Restriction fragment length polymorphism.
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SBP	Systolic blood pressure.
SHR	Spontaneously hypertensive rats
SMC	Smooth muscle cell
SNPs	Single nucleotide polymorphisms.
SOD	Superoxide dismutase
SPSS	Statistical Product and Service Solutions.
SR-BI	Scavenger receptor class B type I
T	Thymine
TBE	Tris-borate-EDTA.
TG	Triglyceride
TNF-α	Tumor necrosis factor-α.
U	Unit.
URL	Upper reference limit.
UV	Ultraviolet.
VCAM-1	Vascular cell adhesion molecule-1
VIP	Vasoactive intestinal peptide
VLDL	Very low density lipoprotein
VSMC	Vascular smooth muscle cell

CHAPTER ONE

GENERAL INTRODUCTION

Hypertension is a substantial public health problem that is a major risk factor for many common causes of morbidity and mortality including stroke, myocardial infarction, congestive heart failure, and end stage renal disease (Mosterd et al., 1999).

In 2006 an official Malaysian report has shown that the prevalence of hypertension among adults (30 years old and above) in Malaysia was 43% (4.8 million Malaysians with hypertension). The estimated figure worldwide is a staggering 1 billion individuals. There is a widely held misconception that hypertension is a single disease that can be treated with a single recipe. Hypertension is a heterogenous disorder in which patients can be stratified by pathophysiologic characteristics. The pathogenesis of hypertension remains largely unknown. It is believed that hypertension occurs from complex interactions between multiple environmental and genetic factors (Lifton et al., 1996). Although much is known about the environmental factors that predispose individuals to hypertension, not much data is known about genetic factors. Genetic factors might constitute between (30 - 60%) of blood pressure variations (Norman et al., 2011).

It is also clear from the evidence in the literature that oxidative stress plays an important role in the pathogenesis of hypertension (Touyz et al., 2004). There were evidences suggesting that apoE and PON-1 proteins exhibit a significant antioxidant activity in the human body. Polymorphisms within the apoE and PON-1 genes have also been strongly linked with the formation of atherosclerosis and other various

pathologies apart from their known physiological function including dementia and infectious diseases (Marta et al., 2004).

Apolipoprotein E (apoE) is a polymorphic multifunctional protein. It is coded by three alleles (Ɛ2, Ɛ3, and Ɛ4) of a modulator gene at the apoE locus on chromosome 19, determining six apoE genotypes and plasma phenotypes (Mahley, 1988). Its pleiotropic effects are exerted on plasma lipoprotein metabolism, coagulation, oxidative processes, macrophage, glial cell and neuronal cell homeostasis, adrenal function, central nervous system physiology, inflammation, and cell proliferation (Davignon et al., 2002). ApoE polymorphism modulates susceptibility to many diseases. It is, however, particularly notorious for its role in neurodegenerative disorders and atherosclerotic arterial disease (Davignon et al., 1988). The Ɛ4 allele (phenotypes Ɛ4/4 and Ɛ4/3) that is associated with higher low density lipoprotein cholesterol (LDL) is considered proatherogenic, whereas the presence of the Ɛ2 allele (E3/2, E2/2), being associated with lower LDL levels, is deemed to have the opposite effect although it might be associated with increased plasma triglycerides and lipoprotein remnants (Mahley & Rall, 2000).

Paraoxonase 1 (PON-1) is a calcium-dependent enzyme which functions as an esterase and lactonase. PON-1 is a member of a three-gene family: PON2, PON3, and PON-1, located on the long arm of human chromosome 7 (Humbert et al., 1993). PON-1 is primarily synthesized in the liver and then anchored to HDL particles in the circulation (Blatter et al., 1993). The primary physiological role of PON-1 is to inhibit LDL oxidation thus it alteres the development of atherosclerosis (Marta et al., 2004). In addition PON-1 enzyme also hydrolyzes organophosphorous compounds, the information that has generated much interest in the field of toxicology (Draganov et al., 2004). There are two important polymorphisms in the coding region of PON-1

gene. These polymorphisms are located at positions 192 and 55. Glutamine (Q) to arginine (R) substitution at codon 192 is the most studied polymorphism. It results in different hydrolytic activity of the alleles towards various substrates. The other important polymorphism at position 55 has no effect on the enzyme activity (Humbert el al., 1993). The Q allele of codon 192 is more efficient towards oxidized high-and low-density lipoproteins (ox-HDL and ox-LDL) than the R allele. It was suggested that the R alloenzyme was less efficient in protecting lipoproteins from oxidation, and may contribute to one risk of developing cardiovascular diseases (Aviram et al., 2000).

The relation of apoE and PON-1 192Q/R genes polymorphism with hypertension is controversial and very few studies tried to illuminate such an issue. The fact that products of these two genes are important in protection against oxidative stress (Marta et al., 2004) and with the understanding that essential hypertension individuals were prone to oxidative stress (Prabha et al., 1990), it is hypothesized that defect within these genes may partly contribute to hypertension development.

CHAPTER TWO

LITERATURE REVIEW

2.1 HYPERTENSION

2.1.1 Overview

Hypertension is a chronic medical disorder associated with high morbidity and mortality. It is characterized by an elevated systemic blood pressure (BP). A person who has sustained systolic BP of greater than 140 mm Hg and/or a diastolic BP at greater than 90 mm Hg is considered to be a hypertensive patient (Kannel, 1996).

2.1.2 Definition of Blood Pressure and High Blood Pressure

Blood pressure (BP) is the pressure exerted by circulating blood upon the walls of blood vessels, and is one of the principal vital signs. During each heartbeat, BP varies between a maximum (systolic) pressure during cardiac contraction and ejection and a minimum (diastolic) pressure during cardiac relaxation as shown in figure 2.1.

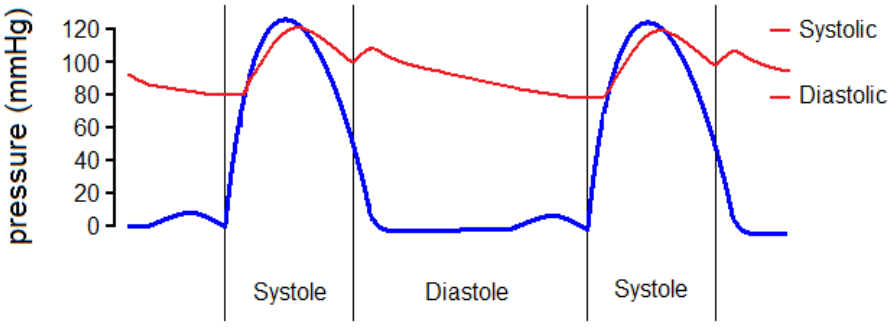

Figure 2.1: Pressure changes in the aorta (red line) and left ventricle (blue line) during systole and diastole (Guyton 2006).

According to the 2007 report of the Malaysian society of hypertension (Malaysian society of hypertension, 2011), BP can be classified into optimal, normal, and prehypertension, while hypertension is divided into; hypertension grade I, grade II, and grade III as shown in table 2.1.

Table 2.1
Classification of hypertension

Classification of Blood Pressure	Systolic BP (mmHg)	Diastolic BP (mmHg)
Optimal	< 120 and	< 80
Normal	120-129 and/or	< 85
Prehypertension	130-139 and/or	85-89
Hypertension grade I (mild)	140-159 and/or	90-99
Hypertension grade II (moderate)	160-179 and/or	100-109
Hypertension grade III (sever)	≥ 180	≥ 109
Isolated systolic hypertension grade I	140-149	< 90
Isolated systolic hypertension grade II	≥ 160	< 90

The threshold BP levels for the diagnosis of hypertension using self/home monitoring devices are when the BP is greater than 135/85 mmHg. In 24 – hour ambulatory BP monitoring, a BP of greater than 125/80 mmHg is considered as hypertension.

2.1.3 Blood Pressure Measurement

Blood pressure is measured by the use of the sphygmomanometer. At least two measurements spaced by 1 to 2 minutes per visit are recommended (Mancia et al., 2007). The 24-hour ambulatory BP measurement (ABPM) is another method of BP measurement which has shown that BP is highest during the day and lowest during the night in both normotensive and hypertensive subjects. Patients with essential hypertension are divided into two groups based on circadian BP patterns: dippers (DH) and non-dippers (NDH). The dippers manifest a reduction of BP during the night, and non-dippers exhibit persistently elevated BP throughout the 24-hour period (Niiranen et al., 2006). Compared with dippers, non-dippers have higher left ventricular mass and higher cardiovascular morbidity (Verdecchia et al., 1994).

2.1.4 Etiology and Risk Factors of Hypertension

2.1.4.1 Causes of Hypertension

The majorities (80–90%) of patients with hypertension have primary elevation of BP, i.e. essential hypertension of unknown cause, while secondary hypertension is where BP elevation is the result of a specific and potentially treatable cause.

Secondary forms of hypertension include renal diseases which accounts for over 80% of the cases of secondary hypertension. The renal causes of hypertension include diabetic nephropathy, chronic glomerulonephritis, adult polycystic disease, and renovascular disease. The mechanism of this BP elevation is primarily due to sodium and water retention, although there can be inappropriate elevation of plasma renin levels (Carretero et al., 2000).

The remaining secondary causes of hypertension include endocrine diseases, drug induced hypertension and congenital hypertension. The endocrine causes

including: Conn's syndrome, adrenal hyperplasia, phaeochromocytoma, Cushing's syndrome, acromegaly. Coarctation of the aorta is a congenital cause of hypertension. Drugs like NSAIDs, oral contraceptives, steroids, sympathomimetics and vasopressin may cause secondary elevation of systemic blood pressure (Manolis et al., 2010).

2.1.4.2 Risk Factors for Hypertension

Non modifiable risk factors for hypertension include age as elderly people are more susceptible to hypertension. Another non modifiable risk factor is gender, as females are prone to hypertension in younger age groups due to pregnancy induced hypertension. (Kumar & Clark, 2009).

Evidences of genetic risks for hypertension came from previous family studies, where children of hypertensive parents tend to have higher BP than age-matched children of parents with normal BP (Hamilton et al., 1954).

Environmental conditions that are associated with higher risk for hypertension includes obesity, sedentary life style, alcohol intake, tobacco smoking, high sodium intake, low potassium intake, and chronic stress (Appel et al., 1997).

2.1.5 Prevalence of Hypertension

In 2000 it was estimated that nearly one billion people or ~26% of the adult population had hypertension worldwide (Kearney et al., 2005). Hypertension was common in both developed (333 million) and undeveloped (639 million) countries (Kearney et al., 2005). Hypertension rates vary markedly in different regions with rates as low as 3.4% (men) and 6.8% (women) in rural India and as high as 68.9% (men) and 72.5% (women) in Poland (Kearney et al., 2004).

In Malaysia the prevalence of hypertension among Malaysians above the age of 30 years was 43% that is 4.8 million Malaysian hypertensives as published by the Third National Health and Morbidity Survey in 2006, unfortunately only 36% of them were aware they had hypertension (Maskon et al., 2010).

2.1.6 Morbidity and Mortality

Hypertension is the most important cause for death in industrialized countries (Novo et al., 2009). It increases hardening of the arteries (Riccioni et al., 2009) thus predisposes individuals to heart disease that includes myocardial infarction, heart failure and left ventricular hypertrophy. Hypertension also predisposes patients to both hemorrhagic and ischaemic strokes. An extreme BP may lead to hypertensive encephalopathy. Other complications include hypertensive retinopathy, hypertensive nephropathy and peripheral vascular disease (Kumar & Clark, 2009).

2.1.7 Physiological Regulation of Blood Pressure

According to the duration by which the body responds to changes in blood pressure, there are two types of control mechanisms. The nervous control system which responds rapidly to pressure changes (over seconds). While the renal and fluid and electrolyte factors are responsible for long-term regulation of the arterial pressure (Britton et al., 1949).

The regulation centre in the brain is represented by the vasomotor centre which consists of groups of neurons situated in the medulla oblongata. When excited the vasomotor discharge is increased causing an increase in heart rate and contractility, increased arteriolar constriction and venoconstriction and a decrease in the stores of blood in the venous reservoirs thus will eventually cause an increase in the mean