ANGIOTENSIN II TYPE 1 RECEPTOR GENE $A^{1166}C$ POLYMORPHISM IN HYPERTENSION; A STUDY ON ITS INFLUENCE ON AORTIC STIFFNESS AND RESPONSE TO ANTIHYPERTENSIVE THERAPY AMONG MALAYS

by

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DEDICATION

In the precious memories of

Professor Dr. Muhammad Jaffar Rehman
Maryam Rehman
Humaira Rehman
Bushra Rehman
Halima Rehman
Hamna Jaffar

To all those who made my life worthwhile

My husband and my daughters
Tariq
Khadeeja & Huda

All my siblings
&
My mother
Mrs. Abdul Ghafoor

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ACE	Angiotensin I converting enzyme
ACEI	Angiotensin I converting enzyme inhibitors
ADI	Autosomal dominant inheritance
AGT	Angiotensinogen
AI	Augmentation index
AME	Apparent mineralocorticoid excess
Ang I	Angiotensin I
Ang II	Angiotensin II
ARB	Angiotensin II receptor blocker
ARI	Autosomal recessive inheritance
AS	Aortic stiffness
AT_1	Angiotensin II type 1
AT1R	Angiotensin II type 1 receptor
AT_2	Ang II type 2
ATP	Adenosine triphosphate
ATPase	Adenosine triphosphatase
baPWV	Brachial ankle pulse wave velocity
BMI	Body mass index
bp	Base pairs
BP	Blood pressure
BSA	Bovine serum albumin
CAD	Coronary artery disease
CAH	Congenital adrenal hyperplasia

CCB	Calcium channel blockers
cDNA	Complementary deoxyribonucleic acid
CEI	Converting enzyme inhibitor
CF	Carotid femoral
CHD	Coronary heart disease
CI	Confidence interval
cm	Centimetres
CRF	Case report form
CTU	Clinical trial unit
CV	Cardiovascular
cv	Coefficient of variation
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
ddH_2O	Double distillied water
dH_2O	Distilled water
DNA	Deoxyribnucleic acid
ECG	Electrocardiogram
EDTA	Ethylene diamine tetra acetic acid
eNaC	Epithelial sodium channel
EtBr	Ethidium bromide
FBC	Full blood count
FBS	Fasting blood sugar
Fig.	Figure
GFR	Glomerular filtration rate
GLP	Good laboratory practice

GRA	Glucocorticoid remediable aldosteronism
HCl	Hydrochloride
HR	Heart rate
HSD	Hydroxysteroid dehydrogenase
HUSM	Hospital Universiti Sains Malaysia
I/D	Insertion or deletion
IHD	Ischemic heart disease
IMT	Intima media thickness
IP	Intermediate phenotype
ISH	Isolated systolic hypertension
JNC	Joint National Committee for the Detection, Evaluation and Treatment of High Blood Pressure
kb	Kilo base
LVH	Left ventricular hypertrophy
LVM	Left ventricular mass
LVMI	Left ventricular mass index
M	Molar
M0	Baseline visit
M1	One month post treatment visit
M4	Four months post treatment visit
MAP	Mean arterial pressure
MI	Myocardial infarction
MLR	Multiple linear regression
MR	Mineralcorticoid receptor
NHMS2	Second National Health and Morbidity Survey

NO	Nitric Oxide
OD	Optical density
PCR	Polymerase chain reaction
PHS	Pseudohypoaldosteronism
PP	Pulse pressure
PS	Power and sample size
PWV	Pulse wave velocity
RAAS	Renin angiotensin aldosterone system
RCLB	Red cell lysis buffer
RCT	Randomized controlled trial
RE	Restriction enzyme
RFLP	Restriction fragment length polymorphism
RFT	Renal function test
RNA	Ribonucleic acid
rpm	Revolutions per minute
SBP	Systolic blood pressure
SD	Standard deviation
SDS	Sodium dodecyl sulphate
SLR	Simple linear regression
SNPs	Single nucleotide polymorphisms
SOP	Standard operating procedures
STE	Sodium tris-EDTA
TBE	Tris-Borate EDTA
TE	Tris-EDTA
Tm	Melting temperature

USM	Universiti Sains Malaysia
UV	Ultraviolet
VSMC	Vascular smooth muscle cells
W /H	Waist hip ratio
WBC	White blood cells
WHO	World health organization
WHR	Waist hip ratio

Angiotensin II type 1 gene reseptor $A^{1166}C$ polimorfisme; kajian terhadap kesanya ke atas keteganan salur darah besar dan tindak balasnya terhadap terapi antihypertensi di kalangan populasi Melayu yang manghadapi darah tinggi

ABSTRAK

Hipertensi merupakan penyumbang utama kepada penyakit kardiovaskular yang merupakan penyebab utama kematian di Malaysia. Ketegangan pembuluh darah besar merupakan penanda kepada kecacatan dan kematian kardiovaskular di kalangan pesakit-pasakit ini. Polimorfisme gen reseptor Angiotensin II jenis I, $A^{1166}C$ telah dibuktikan mempunyai kaitan dengan "essential hypertension" dan ketegangan pembuluh darah besar yang diukur sebagai kelajuan gelombang nadi (PWV). Rawatan menggunakan perindopril iaitu enzim perencat penukaran Angiotensin (ACEI) didapati dapat menurunkan PWV di kalangan penghidap hipertensi yang membawa alel C^{1166}. Data yang menunjukkan kaitan di antara alel $A^{1166}C$ bagi gen AT1R dengan hipertensi di kalangan penduduk Asia adalah bercanggah, hanya sedikit sahaja yang diketahui tentang kaitannya dengan PWV dan pengaruhnya ke atas tindakbalas perawatan anti-hipertensi. Kajian ini dilakukan untuk menentukan (1) hubungan di antara polimorfisme C^{1166} gen AT1R dengan hipertensi dan PWV di kalangan subjek Melayu yang normal serta yang menghidap hipertensi dan (2) untuk mengkaji pengaruhnya ke atas PWV dengan membandingkan penggunaan dua penghalang sistem renin-angiotensin-aldosteron.

Kajian secara keratan rentas dilakukan ke atas seramai dua ratus satu orang subjek yang menghidap hipertensi tanpa sebarang komplikasi kardiovaskular dan juga dua ratus satu orang subjek normal yang telah dipadankan mengikut umur dan jantina. Tekanan darah (BP), PWV, pengukuran antropometrik (tinggi, berat, ukurlilit pinggang dan pinggul) dicatatkan dan nisbah ukurlilit pinggang

terhadap pinggul dan indeks jisim badan (BMI) dikira. Sampel darah vena diambil untuk ujian makmal rutin dan analisa genetik. Polimorfisme $A^{1166}C$ dikesan melalui tindakbalas rantaian polimerase diikuti dengan pemotongan oleh endonuklease penyekat (restriction endonuclease). Bahagian kedua kajian ini melibatkan seramai 46 orang subjek hipertensi tanpa polimorfisme C^{1166} bagi gen AT1R dan tanpa sebarang kerosakkan ke atas organ sasaran, telah ditentukan sacara rawak untuk menerima samaada perindopril atau losartan dengan kajian selari rabun dua belah selama empat bulan dan selepas tempoh "washout" selama dua minggu. Sepanjang tempoh kajian dilakukan, sukatan ubat yang diberikan telah disesuaikan untuk mancapai tekanan darah yang telah ditetapkan (<140/90mmHg) dan sekiranya perlu indapamide 1.5mg akan diberikan bersama dengan ubatan kajian. Kadar denyutan jantung, tekanan darah sistolik dan diastolic (SBP dan DBP) serta PWV diukur di tahap awal, selepas tempoh sebulan dan empat bulan kajian. Dalam kedua-dua kajian, PWV diukur menggunakan mesin Complior®. Data daripada kedua-dua kajian dianalisa menggunakan program statistik (SPSS 11.0) yang bersesuaian.

Keputusan yang diperolehi daripada kajian 1 menunjukkan frekuensi alel C^{1166} di kalangan pesakit hipertensi adalah 7.96% dan sebanyak 7.73% di kalangan subjek normal oleh itu frekuensi alel C^{1166} adalah tinggi sedikit di dalam populasi yang menghidap hipertensi di mana kesignifikan yang ditujukkan adalah pada tahap sempadan (p=0.091). Tiada perbezaan yang signifikan bagi SBP dan DBP di antara pembawa alel C^{1166} dengan bukan pembawa bagi kumpulan hipertensif (p=0.09 dan p=0.161, masing masing). Begitu juga tiada perbezaan signifika bagi SBP dan DBP bagi kumpulan normotensif (p=0.708

dan p=0.838, masing masing) dan di antara populasi kajian keseluruhannya bagi SBP dan DBP (p=0.174 dan p=0.431, masing masing). Subjek yang membawa alel C^{1166} menunjukkan bacaan PWV yang tinggi sedikit berbanding dengan bukan pembawa di dalam kumpulan hipertensif (11.09 ± 2.08 berbanding 10.72 ± 1.80; p=0.093) yang mana ia menunjukkan kesignifikan pada tahap sempadan. Tiada perbezaan pada PWV di kelangan pembawa dan pembawa di antara kumpulan normotensif (9.86 ± 1.18 berbanding 9.53 ± 1.54; p=0.440). Walau bagaimanapun apabila kedua-dua kumpulan normotensif dan hipertensif dianalisa bersama didapati pembawa alel polimorfisme C^{1166} menunjukkan PWV yang lebih tinggi dan bermakna berbanding dengan yang tanpa polimorfisme (10.52 ± 1.82 berbanding 10.15 ± 1.80; p=0.040). Di dalam kajian II, sejumlah 19 orang pesakit hipertensi diberikan losartan dan sejumlah 20 orang pula diberikan perindopril telah berjaya menamatkan kajian. Subjek bagi kedua-dua kumpulan dipadankan dari segi umur, pengukuran antropometrik dan jantina. Tiada perbezaan yang signifikan dicerap untuk tekanan darah yang diukur pada tahap awal di antara kedua-dua kumpulan (150.89 ± 13.91 / 93.68 ± 10.37 berbanding 151.85 ± 12.21 / 91.65 ± 7.54; p=0.821 dan 0.486) dan bacaan PWV (11.63 ± 1.75 berbanding 10.97 ± 1.69; p=0.293). Selepas empat bulan rawatan terdapat penurunan yang signifikan berbanding tahap awal pada SBP (13.57 ± 15.97; p=0.002), DBP (8.26 ± 8.54; p=0.001) dan bacaan PWV (0.83 ± 1.19; p=0.007) bagi kumpulan yang mengambil losartan dan; SBP (17.95 ± 12.26; p<0.001), DBP (9.25 ± 6.23; p<0.001) dan PWV (0.57 ± 1.22; p=0.047) bagi kumpulan yang mengambil perindopril. Walau bagaimanapun, tiada perbezaan yang signifikan dicerap bagi penurunan tekanan darah sistolik (p=0.342) dan diastolik (p=0.681) serta

bacaan PWV ($p=0.521$) di antara kedua-dua kumpulan di kalangan subjek Melayu tanpa polimorfisme C^{1166} yang menghidap hipertensi. Analisis regresi menunjukkan bahawa penurunan di dalam bacaan PWV di antara kumpulan yang mengambil losartan dan perindopril adalah tidak bersandaran dengan penurunan tekanan darah disebabkan oleh pengambilan ubat-ubat ini ($p<0.05$) dan penurunan tekanan darah hanya menyumbang sebanyak 22% ($r^2=0.221$) bagi kumpulan yang mengambil losartan dan 21% ($r^2=0.209$) bagi kumpulan yang mengambil perindopril, daripada jumlah perbezaan keseluruhan di dalam PWV.

Hasil kerja kajian ini menunjukkan bahawa frekuensi polimorfisme C^{1166} adalah hampir sama di antara subjek Melayu yang menghidap hipertensi dan yang normal dan ianya tidak berkaitan dengan kejadian hipertensi itu sendiri. Polimorfisme $A^{1166}C$ didapati tidak berkait dengan PWV di kalangan pesakit hipertensi dan subjek normal tetapi menunjukkan perkaitan yang signifikan dengan PWV di kalangan populasi Melayu keseluruhannya. Di kalangan subjek Melayu yang menghidap hipertensi tetapi tidak membawa polimorfisme ini, rawatan menggunakan losartan dan perindopril selama empat bulan menunjukkan penurunan yang signifikan dan hampir sama dari segi bacaan BP dan PWV. Penurunan bacaan PWV oleh losartan dan perindopril adalah separa bersandaran dengan kesannya ke atas penurunan tekanan darah.

Kata kunci : Hipertensi, ketegangan pembuluh darah besar, reseptor gen angiotensin II jenis 1, kelajuan gelombang denyutan nadi, Melayu, ubatan anti-hipertensi.

Angiotensin II type 1 receptor gene $A^{1166}C$ polymorphism in hypertension; a study on its influence on aortic stiffness and response to antihypertensive therapy among Malays

ABSTRACT

Hypertension is a major contributor to cardiovascular disease (CVD) which is the leading cause of death in Malaysia. Aortic stiffness (AS) is an independent marker of cardiovascular (CV) morbidity and mortality in these patients. Angiotensin II type 1 receptor (AT1R) gene $A^{1166}C$ polymorphism has been shown to be associated both with essential hypertension and AS measured as pulse wave velocity (PWV). Treatment with angiotensin converting enzyme inhibitor (ACEI) perindopril has been shown to reduce PWV among hypertensive patients carrying C^{1166} allele. Data on association of AT1R gene $A^{1166}C$ allele with hypertension among Asians is controversial, while little is known about its association with PWV and its influence on response to antihypertensive treatment. Studies in this thesis were done to determine (1) the association between C^{1166} polymorphism of AT1R gene with hypertension and PWV among Malay hypertensive and normotensive subjects and (2) to study its influence on reduction in PWV comparing two blockers of the renin angiotensin aldosterone system.

Two hundred and one hypertensive without evidence of CV complication and 201 age and sex matched normotensive subjects were studied in a cross sectional design. Blood pressure (BP), PWV, anthropometric measurements (height, weight, hip and waist circumference), were recorded and waist hip ratio and body mass index (BMI) were calculated. Venous blood samples were obtained for routine laboratory investigations and genetic analysis. $A^{1166}C$ polymorphism was detected by polymerase chain reaction followed by

restriction endonuclease digestion. In a second study 46 hypertensive subjects without C^{1166} polymorphism of AT1R gene and without evidence of target organ damage, were randomly assigned to receive either perindopril or losartan in a double blind parallel fashion for 4 months after a washout period of two weeks. During the study, dose was adjusted to achieve target blood pressure (<140/90 mmHg) and if required indapamide 1.5 mg was added to the study medication. Heart rate, systolic and diastolic blood pressure (SBP and DBP) and PWV were measured at the baseline, one month and 4 months after treatment. In both studies PWV was measured using automated Complior® machine. Data from both studies was analyzed using statistical software (SPSS 11.0) using appropriate tests.

Results from study I showed that C^{1166} allele frequency was 7.96% among hypertensive patients and 7.73% among the normotensive subjects. There was therefore a slightly higher C^{1166} allele frequency in the hypertensive population which was of borderline significance ($p = 0.091$). There was no significant difference in SBP and DBP between carriers and non carriers of C^{1166} allele in hypertensive group ($p=0.09$ and $p=0.161$, respectively). Likewise there was not significant difference in SBP and DBP in the normotensive group ($p=0.708$ and $p=0.838$, respectively) and in the overall study population ($p=0.174$ and $p=0.431$, for SBP and DBP respectively). Subjects carrying C^{1166} allele had slightly higher PWV as compared to non carriers in the hypertensive group (11.09 ± 2.08 vs. 10.72 ± 1.80; $p = 0.093$) which was also of borderline significance. No difference in PWV was seen among carriers and non carriers in the normotensive group (9.86 ± 1.18 vs. 9.53 ± 1.54, $p = 0.440$). However

when both normotensives and hypertensives were analyzed together, C^{1166} polymorphism carriers had significantly higher PWV as compared to those without this polymorphism (10.52±1.82 vs. 10.15±1.80, p= 0.040). In study II, a total of 19 hypertensive patients on losartan and 20 on perindopril completed the study. In both the groups patients had similar age, anthropometric measurements and sex distribution. There was no significant difference in baseline BP (150.89 ± 13.91/ 93.68 ± 10.37 vs. 151.85 ± 12.21/ 91.65 ± 7.54, p = 0.821 and 0.486) and PWV (11.63 ± 1.75 vs.10.97 ± 1.69, p =0.293) between the groups. After 4 months treatment there was a significant reduction from baseline in SBP (13.57 ± 15.97, p = 0.002), DBP (8.26 ± 8.54, p = 0.001) and PWV (0.83 ± 1.19, p=0.007) in the losartan group and SBP (17.95 ± 12.26, p <0.001), DBP (9.25 ± 6.23, p <0.001) and PWV (0.57 ± 1.22, p = 0.047) in the perindopril group. However there was no significant difference in reduction in SBP (p=0.342), DBP (p = 0.681) and PWV (p = 0.521) between the two groups among Malay hypertensive subjects without C^{1166} polymorphism. Regression analysis showed that reduction in PWV by losartan and perindopril group was independent of reduction in BP by these drugs and reduction in BP explained about 22 % (r^2 = 0.221) in losartan group and 21% (r^2 = 0.209) in perindopril groups, of the total change in PWV.

Work from this thesis shows that the frequency of C^{1166} polymorphism is similar among Malay hypertensive and normotensive subjects and it is not associated with hypertension. $A^{1166}C$ polymorphism is not associated with PWV in hypertensive patients and normotensive subjects but is significantly associated with PWV in the overall Malay population. Among Malay hypertensive subjects

CHAPTER 1
INTRODUCTION

1.1 Hypertension, cardiovascular disease and its impact

Cardiovascular disease (CVD) is the leading cause of death worldwide. According to 2003 report by the world health organization (WHO, 2003) most deaths in today's world are due to non-communicable diseases (33.2 million) and over half of these (16.7 million) are the result of CVD. WHO has therefore called CVD "a neglected global epidemic" and has highlighted the growing severity of this problem (WHO, 2003). In the past CVD has been considered a disease of civilization and high socioeconomic status as it was associated with modern life style and dietary habits. Today CVD is seen with alarmingly increasing prevalence in the developing countries as well (Forrester *et al.*, 1998). In these countries where infectious disease was the main cause of death, a rapid increase in CVD related mortality and morbidity has become an obstacle to both social and economic development. The burden of deaths and disability in developing world caused by cardiovascular conditions now outweighs that imposed by long standing communicable diseases (WHO, 2003). In fact CVD causes twice as many deaths in developing countries as in developed countries where it is now on the decline largely due to better and improved primary prevention and treatment.

According to the world health report in 2001, the number of deaths in South Asian region due to CVD was 5829 which was the highest in the world at that time (WHO, 2001). This figure has increased to 6078 deaths per year according to 2003 world health report (WHO, 2003). To aggravate the problem there is a relatively earlier onset of the disease among Asian people as compared to the western world (Singh *et al.*, 2000). In Malaysia CVD is the principal cause of

morbidity and mortality and constitutes a significant burden on the national resources. In 1992 alone it caused 30% of the total 40% deaths medically recorded in the country. The majority were due to ischemic heart disease (IHD). According to 1995 health survey it was the fourth cause of hospitalization ranking only after pregnancy, labour and accidents but still the top cause of mortality in the country (Malaysia, 1995). In 1997 and 1998, CVD maintained the same rank but the number of deaths increased from 3497 in 1995 to a total of 4,248 deaths. In 1999 a more comprehensive review of the statistics revealed that the death rate reported only in government hospitals due to CVD was 26.5 per 100,000 in 1990, 23.59 in 1997, 23.12 in 1999 & 22.88 in 2000. Death rate due to hypertension or hypertension related heart disease was 8.8 per 100,000 in 1990, 8.86 in 1997, 8.71 in 1999 and 7.85 in 2000 (Malaysia, 1999, Malaysia, 2001, Malaysia, 2002). These figures did not include the deaths occurring unreported as well as those in the private hospitals.

A large portion of CVD related morbidity and mortality is a result of vascular complications of hypertension. Hypertension or pathological rise in BP is a major determinant and risk factor for premature deaths due to stroke, coronary artery disease (CAD), cardiac and renal failure. In 1978 WHO reported that hypertension is the most common of CVD and reported that presence of high BP is clearly associated with shorter life expectancy (WHO Geneva, 1978). The positive relationship between BP and CV disease has long been recognized. This relationship is strong, continuous, independent, predictive and etiologically significant for those with or without CAD (Flack *et al.*, 1995, Stamler *et al.*, 1991). In 2003, WHO reported a list of risk factors indicative of individual's

future health status and the foremost among the top 10 risk factors worldwide is raised BP or hypertension. Other risk factors related to life style follow hypertension such as alcohol intake and tobacco use, cholesterol and obesity. All these factors contribute to and interact with high BP to cause CVD disease. Hypertension and its complications such as cerebrovascular disease and hypertensive heart disease are the principal cause of death in Malaysia according to the Second National Health and Morbidity Survey (NHMS2) done in 1996 (Lim *et al.*, 2004).

According to the estimates in NHMS2 the overall prevalence of hypertension among adults aged 30 years and above was 33% (Lim *et al.*, 2004). A higher prevalence of hypertension in older age group and in women has been reported. Out of the total, the majority remained undiagnosed. Only 33% were aware of their hypertension and 23% were currently on treatment. Among those receiving treatment only 6% achieved an optimal control of BP. There was no difference in the mean BP between treated and untreated hypertensive patients (Lim *et al.*, 2004). The prevalence of hypertension has increased from 14.4% in the first national health and morbidity survey (1986 unpublished data) and in earlier small surveys (Kandiah *et al.*, 1980) to 33% reported by NHMS2. The prevalence rates found are among the highest reported in the literature revealing a grave situation. Hypertension and its sequel among Malays was also earlier highlighted by studies done in Singapore (Hughes, 1989).

In NHMS2 (Lim *et al.*, 2004), different geographical regions in Malaysia presented differences in prevalence of the disease, complications, undiagnosed

cases and inadequacies of the treatment. In Kelantan, a higher prevalence of isolated systolic hypertension (ISH) (3.2%) and possible undiagnosed cases of hypertension (19.5%) was recorded. Significantly lower proportions of diagnosed patients were on treatment and the highest number (55.7%) of those stopping the antihypertensive treatment in Malaysia was also reported in Kelantan. According to the report, out of the three main ethnic groups in Malaysia, highest prevalence was seen among the Malays which were in agreement with higher figures for Kelantan, a state predominantly inhabited by Malays.

Clearly such a grave situation calls for increased awareness and implementation of preventive measures as well as adequate treatment in the community. However an efficient prevention and adequate treatment requires complete knowledge of pathogenesis of disease and its complications, right from the cellular and molecular level which is influenced by cultural and genetic factors. The established role of cultural, environmental and genetic factors in aetiology, pathogenesis, and long term prognosis of hypertension positively recommended a study of these factors in different populations and therefore among Malays.

1.2 Definition of hypertension

Hypertension may be defined as "a state of abnormal arterial structure and function associated with endothelial dysfunction, vascular smooth muscle constriction or remodelling, increased impedance to left ventricular ejection and propensity for atherosclerosis often and not always manifested by an elevated

BP" (Cohn, 1998). In clinical practice hypertension is defined as a BP level equal to or greater than 140 / 90 mmHg systolic and diastolic (Sixth report of the Joint National Committee; JNC VI, on prevention, detection, evaluation and treatment of blood pressure, 1997) Malaysian clinical practice guidelines: CPG, 2002 (Zaher *et al.*, 2002). Hypertensive patients may then be classified according to JNC VI BP classification (1997) (table 1.1).

1.3 Aetiology of hypertension

A pathological increase in BP or hypertension can occur as a result of pathology in other organ systems of the body (secondary hypertension) or in the absence of a known cause (primary or essential hypertension). Secondary hypertension only comprises a small portion of all known hypertension cases (5%) while in the majority (95%) a definite cause of hypertension is unknown. Table (1.2) shows a list of causes of secondary hypertension. Essential hypertension on the other hand is a form of hypertension that cannot be attributed to the existence of another problem or disease. Essential hypertension is known to run in families which suggests a role of inheritance in its aetiology.

The influence of inheritance on hypertension and the role of interaction between multiple genes and environment has been proposed (Cruz-Coke, 1981). It has been considered a genetic disease which occurs because of environmental impact (Mendlowitz, 1982). Therefore interindividual differences in BP are explained based on differences between the genetic structures of an individual as opposed to genetic structure of the population. Different individuals may possess different genetic defects in a population (Lifton, 1995). In order for

Table 1.1

Classification of BP for adults aged 18 years and older

JNC VI (1997)

	Blood pressure mmHg		
Category	**Systolic**		**Diastolic**
Optimal	<120	And	<80
Normal	<130	And	<85
High normal	130-139	Or	85-89
Hypertension			
Stage 1	140-159	Or	90-99
Stage 2	160-179	Or	100-109
Stage 3	≥ 180	Or	≥ 110

Table 1.2

Types and causes of secondary hypertension

(Adapted from Kaplan, 2002)

Systolic and diastolic hypertension

Renal
- Renal parenchymal disease
  - Acute glomerulonephritis
  - Chronic glomerulonephritis
  - Chronic nephritis
  - Polycystic disease
  - Diabetic nephropathy
  - Hydronephrosis
- Renovascular hypertension
  - Renal artery stenosis
  - Other causes of renal ischemia
- Renin producing tumours
- Renoprival
- Primary sodium retention (Liddle's syndrome, Gordon's syndrome)

Endocrine
- Acromegaly
- Hypothyroidism
- Hyperthyroidism
- Hypercalcemia (hyperparathyroidism)
- Adrenal disorders
  - Cortical disorders
    - Cushing's syndrome
    - Primary aldosteronism
    - Congenital adrenal hyperplasia
  - Medullary tumours (pheochromocytoma)
- Extra adrenal chromaffin tumors
- 11-β-hydroxysteroid deficiency/inhibition
- Carcinoids
- Exogenous hormones
  - Estrogens
  - Glucocorticoids
  - Mineralocorticoids
  - Sympathomimetics
  - Erythropoietin
- Foods containing tyramine and mono amine oxidase inhibitors
- Coarctation of aorta
- Pregnancy induced
- Neurologic disorders
  - Increased intracranial pressure
  - Sleep apnoea
  - Quadriplegia
  - Acute porphyria
  - Familial autonomia
  - Lead poisoning
  - Guillain Barre' syndrome
- Acute stress (including surgery)
  - Psychogenic hyperventilation
  - Hypoglycemia
  - Burns
  - Alcohol withdrawal
  - Sickle cell crisis
  - After resuscitation
  - Perioperative
- Increased intravascular volume
- Alcohol
- Nicotine
- Cyclosporine, tacrolimus

Systolic hypertension

Increased cardiac output
- Aortic valvular insufficiency
- Atriovenous fistula, patent ductus
- Thyrotoxicosis
- Paget's disease of the bone
- Beriberi

Arterial rigidity

possess different genetic defects in a population (Lifton, 1995). In order for hypertension to be manifest, a single or a number of genetic defects may be present.

Although common, the aetiology and mechanism of essential hypertension is not fully understood (Meredith *et al.*, 2003). Current therapeutic strategy for the disease is only empirical as a result of which may be one reason why a large number of the patients never achieve an optimal response. This not only increases the cost of treatment, incidence of side effects but also leads to a poor patient compliance finally leading to complications of hypertension. This phenomenon is very well documented among Malaysian hypertensive patients as was shown in the NHMS2 stated earlier in this chapter. This scenario is also a consequence of the fact that it is a complex disease in which a multitude of genetic factors, environmental stimuli, physiological systems and biochemical processes interact to influence arterial function, the BP level and therefore a susceptibility to develop hypertension.

Hypertension is associated with abnormal arterial and endothelial function which affects both large and small arteries. In this regard stiffness of large arteries such as aorta and its major branches is particularly relevant as it directly affects heart upstream. Large artery stiffness causes increased left ventricular load and reduced coronary perfusion besides influencing target organs like brain and kidney down stream. Most of the complications of hypertension are associated with arterial stiffness. Reduction in BP in response to antihypertensive drug therapy may or may not be related with an

improvement in arterial function. As a result different drugs cause reduction in BP with or without reduction in CV risk associated with hypertension. It is therefore of interest to understand the basis of hypertension, its essential accompaniment arterial stiffness as well as the influence of drug therapy.

1.4 Structure and function of large arteries

Human arterial system is a complex network of tubes which serves to deliver blood pumped from the heart through smaller arteries and arterioles into organs and tissues of the body. In doing so it has to function as a conduit, transmitting blood to the tissues as well as a cushion smoothing out pulsations caused by intermittent cardiac contractions so as to achieve a steady blood flow to the tissues. This cushioning function depends upon the mechanical properties of the arteries which are determined by the structural components of the arterial wall. Among the three layers of arterial wall i.e. the intima, media and adventitia, tunica media or the intermediate layer is most important in determining the cushion or elastic properties of the artery. The intima consists of a single layer of endothelial cells, a fenestrated sheet of elastic fibres and the internal elastic lamina. Though it does not contribute to the mechanical behaviour of the arterial wall, it is a rich source of substances and signal transduction mechanisms which influence the mechanical function of the arterial wall (Van Bortel *et al.*, 2001).

Tunica media, the main determinant of mechanical properties, is composed of smooth muscle fibres, a connecting molecular grid composed of mucopolysaccharides and variable amount of collagen and abundant elastic

tissue especially in aorta. Vascular smooth muscle cells (VSMC) are known to be of many different types based on their characteristics such as contractile, proliferative, and synthetic or apoptotic (Schwartz and Mecham, 1995). The relative proportion of each of these phenotypes depends upon age, location in vascular tree and the prevailing pathological condition (Van Bortel *et al.*, 2001) like hypertension. Contribution of each of these types, to the overall mechanical behaviour of the arterial wall is however not yet known. The outermost layer adventitia is mainly composed of fibroblasts and collagen and is abundant in central arteries such as the aorta. It is a major determinant of stiffness of large arteries and may be modified through breakdown, cross linking and glycation. These important components of the arterial wall, although present in all the arteries, exhibit differences in their content between central and peripheral arteries which make them elastic or muscular in character. Therefore on the basis of wall structure arteries can be divided into three types; large elastic (conduits) arteries e.g. aorta and its major branches, medium and small sized arteries e.g. radial and its branches and resistance arteries e.g. arterioles or the distributing arteries.

Large elastic arteries such as aorta and its main branches carotid, brachiocephalic and pulmonary arteries have characteristically thick tunica media rich in concentrically arranged lamina of elastic fibres. Within this elastic lamina are smooth muscles, reticular fibres and chondroitin sulphate ground substance. On the other hand the tunica media of the medium sized peripheral arteries is predominantly composed of smooth muscles with some elastic and reticular fibres. Those of arterioles consist of a few layers of smooth muscles

intermixed with collagenous and reticular fibres. It is therefore the elastic lamellae and their ratio with the collagen content of tunica media of the aorta, which is responsible for its elastic nature and determines its buffering or cushion function. The elastic fibres are responsible for normal arterial distensibility and compliance. During systole, it stores the blood pumped by left ventricle which is then delivered to the body during diastole in a continuous stream.

1.5 Arterial function and blood pressure regulation

As a result of the conduit and cushioning functions of arteries, arterial blood pressure has two components, a steady component represented by the mean blood pressure and, a pulsatile component represented by pulse pressure. Mean blood pressure is determined by cardiac output and total peripheral resistance which is in turn determined by the calibre and number of small arteries and arterioles. Pulse pressure on the other hand represents oscillations around the mean pressure, the systole and diastole being the highs and lows of the oscillations. Magnitude of pulse pressure depends on the pattern of left ventricular ejection, the viscoelastic and propagative properties of large arteries and the timing of reflected waves (Safar, 1996). A rise in peripheral resistance causes a proportionate rise in systolic and diastolic pressure therefore mean arterial pressure. On the other hand a reduction in viscoelasticity i.e. increase in stiffness of arterial wall only modifies the shape of blood pressure curve, with systolic and diastolic pressure rising and decreasing respectively, without causing a change in the mean arterial pressure but widening the pulse pressure (Safar, 1996) (fig. 1.1).

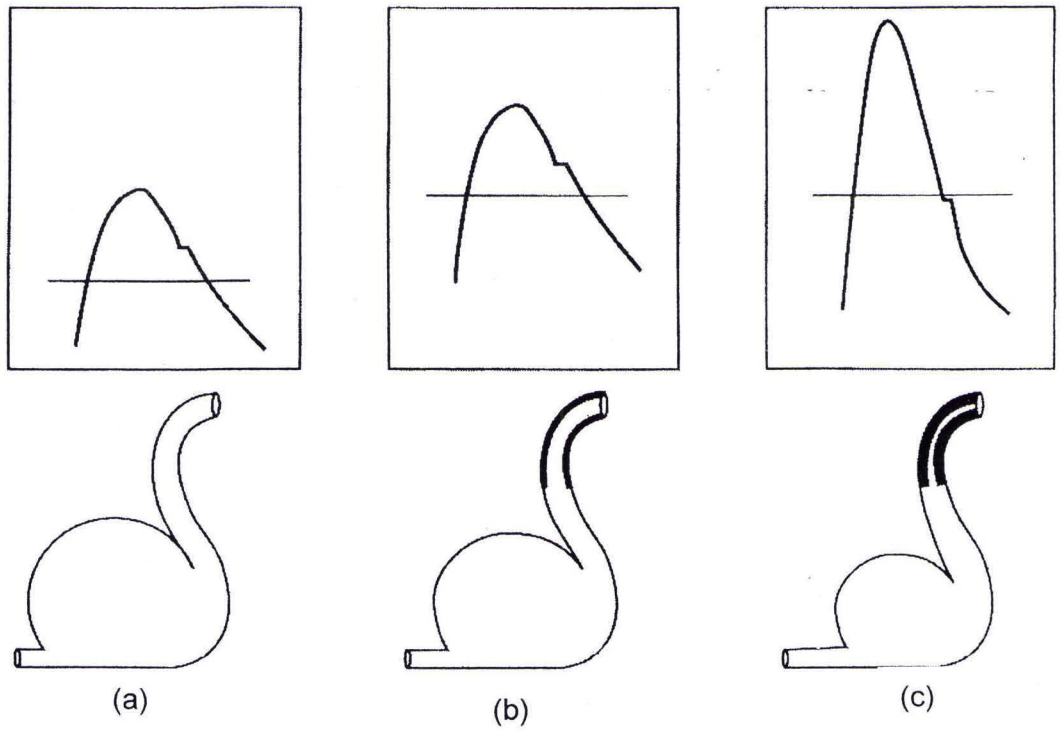

Figure 1.1

Blood pressure curves in compliant vs. stiff arteries

Effect of increased resistance and reduced elastance on blood pressure curve, peak systolic and end diastolic pressure.

a) Distensible artery with normal resistance and normal BP curve
b) Distensible artery with increased resistance and BP curve showing a proportionate increase in systolic and diastolic BP.
c) Stiff artery with increased resistance, BP curve shows a disproportionate rise in systolic BP

(Adapted from Safar, 1996)

Pulse pressure is the difference between systolic and diastolic blood pressure (SBP and DBP). In young healthy subjects, pulse pressure increases significantly from central to peripheral arteries, principally because of a rise in systolic pressure with a relative fall in diastolic pressure. This is due to differences in mechanical properties of central and peripheral arteries as a result of different collagen and elastin content in their tunica media. Carotid artery pulse pressure which is virtually identical to aortic pressure is lower than radial and femoral artery pulse pressure. The highest pressure values are seen in the common femoral arteries. This pressure gradient along the arterial tree is similar in both normotensive and young hypertensive subjects although absolute values are higher in hypertensive patients but diminishes with old age.

In pulsatile hemodynamic studies, factors influencing pulse pressure may be analyzed as the summation of an incident pressure wave originating from the heart and reflected pressure waves returning to the heart from the resistance vessels in peripheral circulation i.e. a forward and a backward wave. Forward wave is influenced by ventricular ejection and stiffening of aorta, while backward reflected wave depends on stiffening of arteries and the site of reflection points i.e. the resistance vessels (Safar, 1996) (fig. 1.2). Arterial wall viscoelasticity is a determining factor for speed of wave propagation across the arterial tree and the timing of wave reflections. Increased stiffness of arteries leads to increased pulse wave velocity (PWV) and reflection sites (i.e. resistance vessels) appear to operate closer to the heart producing an earlier reflection of aortic backward wave during systole instead of diastole. An earlier reflection of backward wave leads to a summation of the forward and backward waves during systole, higher

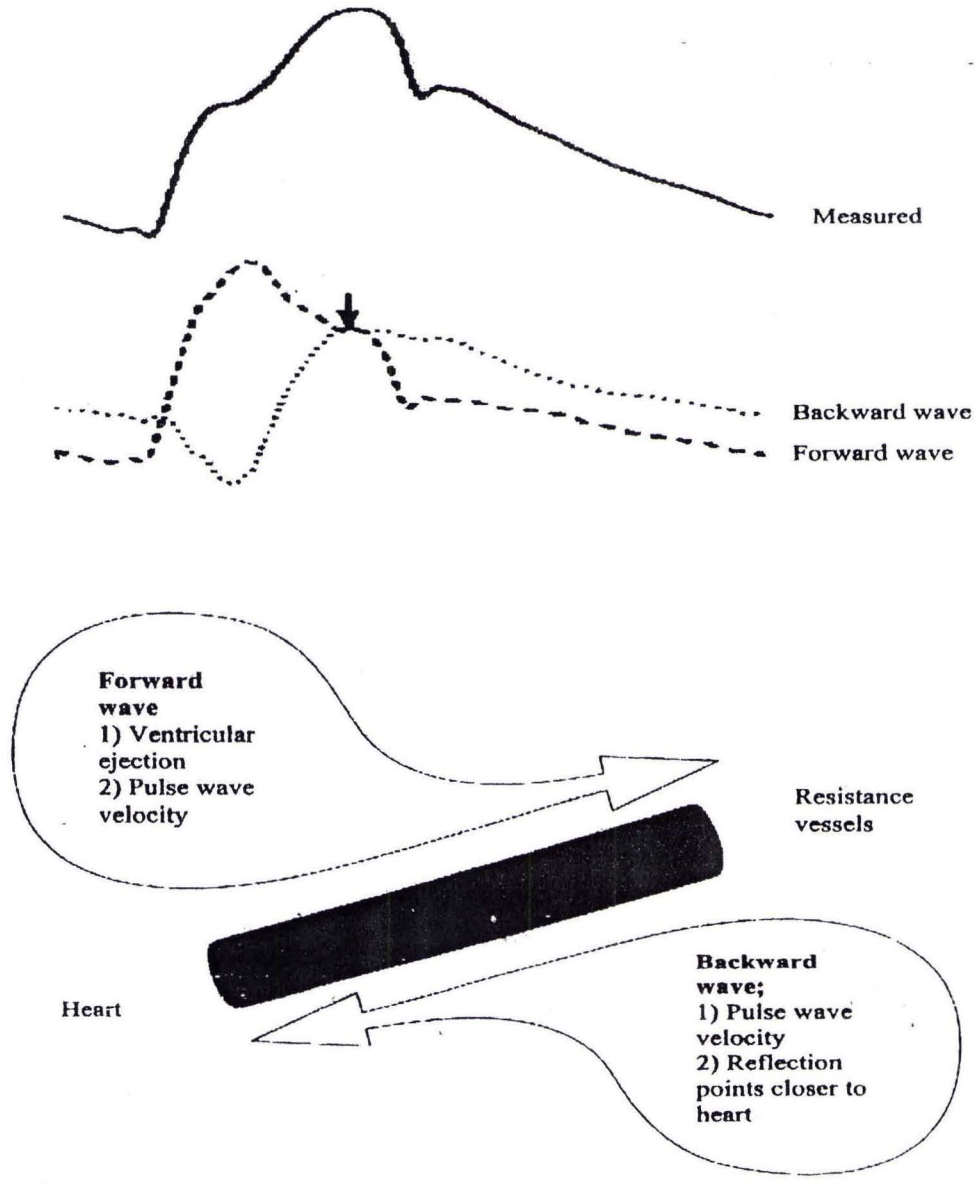

Figure 1.2

Forward and backward waves determining the pulse pressure

Summation curve resulting from the effects of forward and backward waves together with decomposition of the summation curve into forward and backward waves (top).
Factors affecting forward and backward waves determine the pulse pressure and the pulse waveform (bottom)

(Adapted from Safar, 1996).

BP and pronounced systolic peak and systolic pressure (O'Rourke, 1989, Safar and Laurent, 1993). An increase in the systolic arterial pressure accelerates the fatigue of the arterial wall and arterial damage producing a self perpetuating cycle (London and Guerin, 1999). Normally velocity of the pulse wave is low as in young healthy subjects and reflection points are principally observed at the narrowing of small resistance vessels causing a return of reflection wave during diastole.

With aging there is fragmentation and fracture of the elastic lamella followed by fibrous remodelling. Fracture of elastic fibres occurs as a result of repetitive or cyclic stress with each cardiac cycle. The aortic media hence becomes disorganized with faulty areas of mucoid degeneration or medionecrosis as may be seen in elderly or older patients with hypertension (O'Rourke, 1995). As a result, with aging and high BP, increased PWV promotes a disproportionate increase in systolic over diastolic pressure. Therefore for the same mean arterial pressure, pulse pressure is higher in older subjects than younger ones (fig.1.3). For the same reason pulse pressure becomes similar through out the arterial system with disappearance of earlier mentioned pulse pressure gradient between central and peripheral arteries. Stiffness of central elastic arteries as compared to peripheral arteries increases markedly with age. The central pressure increases while the femoral pressure does not change as a consequence of differential increase in stiffness of central over peripheral arteries (Benetos *et al.*, 1991a). Preferential increase in central pressure increases the systolic pressure while there is a relative fall in DBP. Therefore risk of CAD in middle aged and elderly subjects increases with decreasing DBP

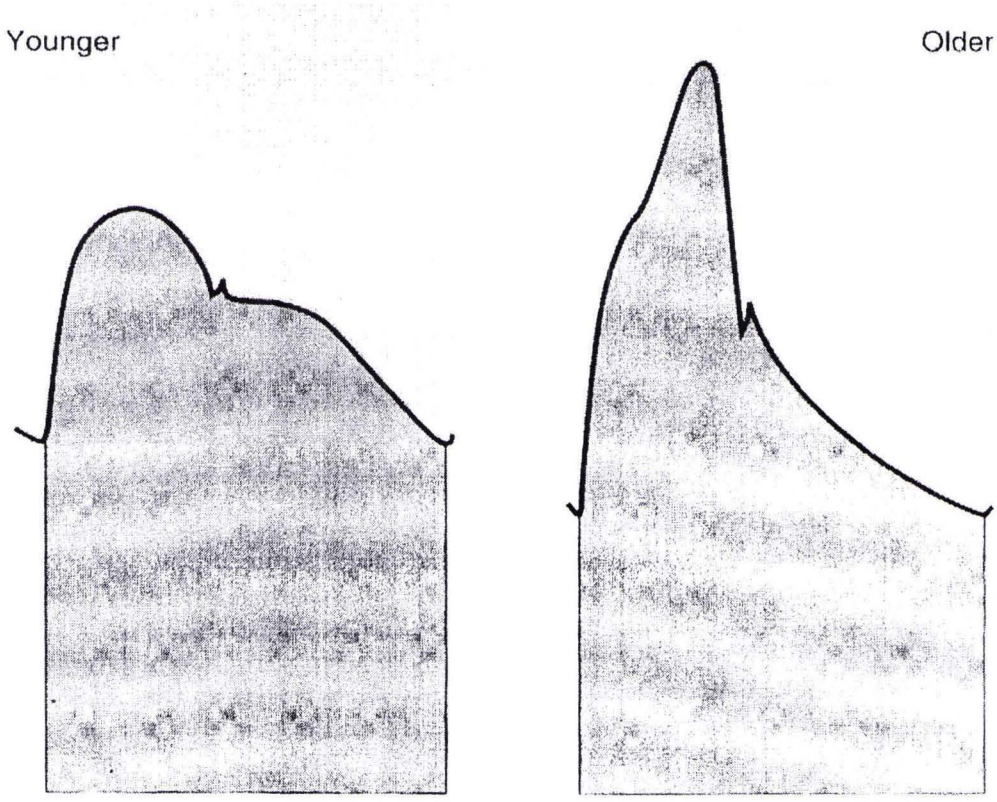

Figure 1.3

BP curves for younger and older subjects.

For similar mean blood pressure, pulse pressure the difference between systolic and diastolic BP is higher in elderly

(Adapted from Safar, 1996).

i.e. widening of the pulse pressure and large artery stiffness (Franklin, 1999a). A wide pulse pressure constitutes a significant predictor of CV events such as myocardial infarction (MI) and is a strong predictor of CV risk even among normotensive subjects (Benetos, 1999).

1.6 Arterial stiffness as an index of arterial function

The principal function of large central arteries like the aorta is its buffering or cushion function and the best clinical index representative of this is arterial compliance. Arterial compliance is defined as "change in volume for a given change in pressure", i.e.

$$C = \Delta V / \Delta P$$

where, C is compliance, ΔV is change in volume and ΔP is change in pressure. In arteries the pressure volume relationship is curvilinear because of variable composition of constituents of arterial wall. As a result arterial compliance is pressure dependent and varies inversely with level of mean arterial pressure within a physiological range. If the artery becomes less distensible, its storage capacity diminishes for any given pressure. Under these circumstances either large fraction of the stroke volume must run off during systole or a greater rise in systolic pressure must occur to accommodate the increased volume in non-compliant or stiff arterial tree. Arterial stiffness therefore has an inverse relationship with arterial compliance, the stiffer the artery the lesser its compliance. Arterial stiffness can therefore serve as an indicator of large arterial function.

Properties like stiffness and compliance are dependent upon blood pressure (O'Rourke and Mancia, 1999). The higher the BP, the lesser the arterial

compliance and greater will be the stiffness. Both elastic and muscular component of arterial wall are essential for the arterial function and its structural integrity. At normal or low levels of pressure as in young healthy subjects, the load is mainly borne by the elastic fibres which are readily stretchable (Glagov *et al.*, 1992). As the pressure and wall stretch increases, the collagen fibres are progressively recruited and the artery behaves as though composed of collagen alone and becomes stiff (Safar, 1996).

1.6.1 Pulse wave velocity as a measure of arterial stiffness

As speed of travel of a wave in any material is determined by the stiffness of that material, arterial stiffness can also be defined in terms of PWV. Pulse wave velocity is therefore an index of arterial stiffness. Newton was the first to show that velocity of wave propagation in a compressible material was determined by its stiffness and density. Later this concept was found to be true in case of arteries in which blood acts as an incompressible material and the energy imparted to the arterial tree with each heart beat travels along the elastic arterial wall. The stiffer the material the faster will be the speed of travel of pulse wave. Pulse wave velocity, measures velocity of energy imparted to the arterial tree by cardiac contractions and is a pure measure of arterial properties (Kelly *et al.*, 1996). The speed of propagation of pulse wave along the vessel wall is much faster than the speed of blood flow within its lumen.

Pulse wave velocity is perhaps the best and most widely used technique for measurement of stiffness of aorta and its major branches (Izzo and Shykoff, 2001). Among the non invasive measures to evaluate the structure and function

of large arteries, it is most simple and accurate (Izzo and Shykoff, 2001). Pulse wave velocity has been used to determine the influence of vasoactive agents (Rehman *et al.*, 2002, Rehman *et al.*, 2001) as well as antihypertensive drugs (Asmar, 2001, Benetos *et al.*, 1996a). Non invasive measurement of PWV using machines such as Complior® has become a technique of choice due to its simplicity and reproducibility and also because it is atraumatic. This measurement technique using the Complior® program has been validated (Asmar *et al.*, 1995a). Carotid femoral PWV indicates stiffness of aorta and has been used in a number of clinical studies as a measure of aortic stiffness (Amar *et al.*, 2001, Asmar *et al.*, 2001a, Blacher *et al.*, 1999, Meaume *et al.*, 2001, Taquet *et al.*, 1993, Yongbin *et al.*, 2003). Aortic stiffness (AS) measured as carotid femoral (CF) PWV is considered the best indicator of CV risk (Izzo and Shykoff, 2001).

1.6.2 Hypertension and arterial stiffness

Hypertension is a major determinant of arterial stiffness second only to aging (Asmar, 1999, Asmar, 2001). Arterial alterations in hypertension occur long before the appearance of any CV symptoms (Shargorodsky *et al.*, 2002). Clinical studies have shown that stiffness of arteries occurs early in hypertension at the time when BP is only elevated to a borderline level of hypertension (Asmar, 1999, Messerli *et al.*, 1985, Simon *et al.*, 1992, Ventura *et al.*, 1984). Increased arterial stiffness has been shown in newly diagnosed and untreated hypertensive subjects (Ngim *et al.*, 1999), middle aged patients with sustained hypertension (Simon *et al.*, 1992) as well as in elderly patients with ISH (Messerli *et al.*, 1982). The age related increase in arterial stiffness is

accelerated in hypertensive patients and has been shown to be augmented in phases according to the severity of hypertension (Tomiyama *et al.*, 2004). Besides these, normotensive offspring of hypertensive patients have been shown to have increased AS (Yasmin *et al.*, 2004).

Increased BP contributes to arterial stiffness in two ways, passive or reversible effects and structural or potentially irreversible effects. Passive and reversible effects of high BP influence both elastic and muscular arteries and are due to passive dilatation of the artery with increased BP. Passive dilatation of arteries due to high BP, recruits collagenous fibres to the elastic fibres already bearing tension on the arterial wall. As a result artery behaves as though stiff (Nichols and O'Rourke, 1990). This passive effect of raised BP is abolished by reduction in BP with antihypertensive agents. In addition to the stretch effect of raised pressure, early changes and abnormalities in the arterial wall structure also contribute to arterial stiffness in hypertension (Asmar, 1999). These structural characteristics are responsible for large artery stiffness and include smooth muscle cell hypertrophy in the medial layer, collagen deposition and dysfunction in proteoglycan metabolism (Benetos *et al.*, 2002, Et-Taouil *et al.*, 2003). Such intrinsic alterations in mechanical properties of arteries are observed more at the site of central arteries like aorta and its proximal branches than the peripheral arteries, and are more pronounced with increasing age (Van Bortel *et al.*, 2001). These changes contribute to vascular remodelling and hypertrophy in hypertension.

Vascular remodelling and hypertrophy in hypertension are due to activation of vasoactive substances like Ang Ii and endothelial dysfunction. Endothelial dysfunction contributing to functional and later structural changes in hypertension (Kung and Luscher, 1995) may vary according to the arterial territory. Endothelial dysfunction is associated with abnormalities in nitric oxide (NO) production and / or release (Van Bortel *et al.*, 2001) and bradykinin-dependent hyper-reactivity of smooth muscle cells and vasa vasorum. Such related mechanisms influence wave reflections and contribute to aortic wall stiffness (Et-Taouil *et al.*, 2003). Improvement in arterial stiffness with antihypertensive drugs such as Ang II type 1 receptor blockers (ARB) has been suggested to be due to correction of endothelial dysfunction through blockade of effects of Ang II (Mahmud and Feely, 2002a).

In short hypertension causes major changes in the arterial pulse through large artery stiffening, increased PWV and early wave reflection (O'Rourke, 1995). There is a close, well known relationship between arterial stiffness and level of BP. At any given ventricular ejection, BP level is determined not only by peripheral resistance i.e. arteriolar constriction but also by the degree of arterial stiffness and resultant changes in amplitude as well as timing of the reflected waves. Therefore the greater the aortic stiffness, the higher will be the pulse velocity with earlier return of the reflected waves during systole instead of diastole. This will lead to a greater augmentation of the systolic pressure (Safar and Frohlich, 1995) and as a result a wider pulse pressure. Increased systolic pressure due to aortic stiffness increases left ventricular afterload and hypertrophy with increased myocardial oxygen demand. Increased myocardial

oxygen demand coupled with reduced coronary perfusion due to relative fall in DBP leads to myocardial ischemia. Hence large artery stiffness in hypertension has deleterious effects on the heart upstream and target organs such as the kidney and the brain downstream by increasing both pulsatile pressure and shear stress. Alterations in large artery function in hypertension are therefore directly related to the classic complications involving central nervous system, heart and kidney and may be observed independent of age and atherosclerotic lesions.

1.6.3 Clinical consequences of arterial stiffness

Large artery stiffness cause major changes in circulation which manifest as systolic hypertension and increased pulse pressure, left ventricular hypertrophy and early atherosclerotic damage. These clinical consequences of large artery stiffness are described below.

1.6.3.1 Systolic hypertension and wide pulse pressure

An increased pulse pressure is the most obvious consequence of aortic stiffness. Increased arterial stiffness contributes to systolic hypertension (Simon *et al.*, 1992) by increasing velocity of the forward pulse waves which reach the reflection points in the arterial tree earlier resulting in earlier return of the reflected wave during systole. It has been noticed that systolic BP increases with age and the diastolic BP increases until 50 years of age and then declines (Safar, 1996). This disproportionate reduction in DBP is mainly due to shift of the reflected pulse waves in systole from diastole as a result of arterial stiffness and is associated with widening of the pulse pressure.

Systolic hypertension occurring consequent to arterial stiffness then interacts with age to further promote arterial stiffness (Izzo and Shykoff, 2001) setting up a self perpetuating cycle.

1.6.3.2 Left ventricular hypertrophy

Left ventricular hypertrophy (LVH) occurs secondary to chronic increase in left ventricular afterload. Cardiac work load is determined by the heart rate, cardiac contractility as well as by systemic vascular resistance and aortic stiffness. Aortic stiffness increases cardiac load not only by generating larger initial systolic pressures but also by an earlier return of the reflected waves. Initially LVH was thought to be the consequence of chronic elevation of BP. However observation of LVH among subjects with normal BP showed that measurement of SBP at the brachial artery misinterprets the central systolic pressure and cardiac afterload, by not taking the effect of reflected waves into account (Izzo and Shykoff, 2001, Simon *et al.*, 1992). On the other hand, strong correlation of measures such as PWV with LVH (Bouthier *et al.*, 1985, Girerd *et al.*, 1991) suggest that arterial stiffness contributes to LVH by an increase in systolic as well as pulse pressures, important determinants of cardiac work load. Both systolic hypertension, with wide pulse pressure and arterial stiffness have been shown to be important CV risk factors (Safar, 2001).

1.6.3.3 Atherosclerosis

In the presence of atherogenic risk factors such as smoking and high cholesterol level, increased large artery stiffness may lead to early