HAEMODYNAMIC EFFECTS OF ADIPONECTIN ON PPAR–γ RECEPTORS IN DIABETIC AND NON-DIABETIC WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS

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by

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DEDICATION

This thesis is dedicated to beloved Prophet Mohammad (peace be upon him). As regards all standards by which human greatness may be measured, we can say it for sure; there is no man greater than Him.

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LIST OF ABBREVIATIONS

%	percentage
µg	micro gram
µL	microliter
µM	micro moles
ACRP	adipocyte complement related protein
ADP	adiponectin
AMPK	adenosine monophosphate-activated protein kinase
ANG II	angiotensin II
ANOVA	analysis of variance
APM1	adipose Most abundant gene transcript1
APPL	adaptor protein containing Pleckstrin homology domain, phosphotyrosine-binding domain and Leucine
ARB	angiotensin receptor blocker
AT_1	angiotensin II (type 1) receptor
ATP	adenosine tri-phosphate
BMI	body mass index
BPU	blood perfusion unit
B.w	body weight
Cl.Cr	creatinine clearance
CRP	C-Reactive Protein
CVD	cardiovascular disease
DM	diabetes mellitus

EDCFs	endothelial-derived constrictors factors
EDRFs	endothelium-derived relaxing factors
ELISA	enzyme-linked immunosorbent assay
eNOS	endothelial nitric oxide synthase
FE_{Na}	fractional sodium excretion
FFA	free fatty acid
g	grams
GBP28	Gelatin binding Protein
GSH	glutathione
HDL	high density lipoprotein
HMW	high Molecular Weight form
HR	heart rate
i.p.	intraperitoneal
Irb	irbesartan
LDL	low density lipoprotein
LMW	low- Molecular Weight
m/s	meter per second
MAP	mean arterial blood pressure
MAPK	mitogen active protein kinase
MDA	malondialdehyde
ME	methoxamine
mg /dl	milligram per deciliter
mg/kg	milligram per kilogram

mL	milliliter
mL/min/kg	milliliter per minute per kilogram
mmHg	millimeter mercury
mRNA	messenger Ribonucleic acid
NA	noradrenaline
NADPH	nicotinamide adenine dinucleotide phosphate oxidase
ng	nano gram
NIBP	non invasive blood pressure
NO	nitric oxide
NOS	nitric oxide synthase
Pcr.	plasma creatinine
PE	phenylephrine
Pio	pioglitazone
PKC	protein kinase C
PPAR	peroxisome Proliferator Activated Receptor
PPAR- -γ	peroxisome Proliferator Activated Receptor gamma
PPAR-α	peroxisome Proliferator Activated Receptor alpha
PPAR-β	peroxisome Proliferator Activated Receptor beta
PWV	pulse Wave velocity
RAAS	renin angiotensin aldosterone system
RCBP	renal cortical blood perfusion
RNA	ribonucleic acid
ROS	reactive oxygen species

SBP	systolic blood pressure
SEM	standard error of mean
SHR	Spontaneously Hypertensive rats
SNS	sympathetic nervous system
T2DM	type 2 diabetes mellitus
T-AOC	total anti-oxidant capacity
TNF-a	tumor necrosis factor-alpha
T-SOD	total superoxide dismutase
TXA2	thromboxane A2
TZDs	thiazolidinedione
U/mL	units per millilitre
Ucr.	urinary creatinine
UFR	urine flow rate
$U_{Na}V$	absolute sodium excretion
VSMCs	vascular smooth muscle cells
WKY	Wistar Kyoto
δ	delta
ε	epsilon

KESAN HEMODINAMIK DARIPADA ADIPONECTIN TERHADAP RESEPTOR PPAR–γ DALAM WISTAR KYOTO TIKUS DIABETES DAN HIPERTENSI SECARA SPONTAN

ABSTRAK

Prevalens hipertensi dan diabetes semakin meningkat dengan kadar yang belum pernah berlaku sebelum ini di kedua-dua negara sedang membangun dan juga di negara maju. Adiponectin, yang merupakan suatu adipokin, adalah hormon protein yang menyederhana tindakannya dengan merangsang pelepasan nitrik oksida (NO) daripada endotelium vascular dan menyebabkan vasodilasi. PPAR-γ merupakan ahli reseptor diaktif proliferator peroksisom, yang merupakan pengawal atur positif bagi ekspresi gen adiponectin. Dalam kajian ini, irbesartan digunakan sebagai agonis PPAR-γ separa, Sebaliknya, pioglitazon bertindak sebagai ligan penuh bagi PPAR-γ dan menyebabkan peningkatan kepekatan adiponectin plasma. Kajian ini mengkaji antihipertensi, antidiabetes, potensi antioksidan dan hemodinamik renal adiponectin dengan agonis PPAR-γ dalam model diabetes dan bukan diabetes daripada tikus hipertensi. Di samping itu, kesan adiponectin dan gabungan rawatan daripada adiponectin dengan agomis PPAR-γ separa atau penuh pada α1-adrenoceptor menyebabkan perubahan vaskular renal dan sistemik daripada tikus hipertensi dan normotensi diabetes dan bukan diabetes turut dikaji. Diabetes jenis I diaruh menggunakan suntikan / injeksi intraperitoneal tunggal daripada streptozotosin pada dos 40 mg/kg berat badan yang dilarutkan dalam penampan natrium sitrat (pH 4.5). Satu set tikus normotensi (WKY) dan hipertensi (SHR) diberikan

irbesartan, pioglitazone, adiponectin dan gabungan adiponectin dengan sama ada irbesartan atau pioglitazon. Sementara itu, satu set lain yang tediri daripada tikus normotensi bukan diabetes (WKY) dan tikus hipertensi (SHR) diberikan pola atau corak rawatan yang sama. Tekanan darah (BP) dan parameter metabolisme diukur pada tikus. Di samping itu, kelajuan gelombang nadi, perfusi darah kortikal renal, adiponectin plasma, profil cecair dan elektrolit juga diukur semasa dan pada akhir kajian. Selanjutnya, tikus diberi natrium pentobarbiton beranestetik dan dikurangkan min tekanan arteri dan aliran darah kortikal renal diaruh melalui pemberian secara sistemik dan intrarenal daripada noradrenalin, fenilefrin, metoksamin dan angiotensin II (Ang II). Data, mean±SEM tertakluk pada ANOVA dengan signifikan pada $P<0.05$. Diabetes SHR mempunyai BP yang lebih tinggi, adiponectin plasma yang rendah, ketidakfungsian renal ditunjukkan dengan peningkatan kreatinin plasma, klearans kreatinin, kumuhan /ekskresi natrium dalam bentuk pecahan dan mutlak. Rawatan dengan adiponectin sahaja dan gabungan adiponectin dengan agonis PPAR-γ separa atau lengkap didapati mengurangkan BP, mengurangkan respons vaskular renal dan sistemik pada agonis α_1-adrenergic dan Ang II, memperbaiki stres oksidatif dan meningkatkan hemodinamik renal dan fungsi kumuhan, justeru, ia menberikan suatu interaksi kompleks di antara subjenis α1-adrenoceptor, reseptor ANG II dan adiponectin reseptor. Gabungan terapi adiponectin dengan pioglitazone menggariskan peranan perlindungan reno daripada adiponectin . Oleh itu, darjah sinergisme wujud di antara adiponectin dan agonis PPAR-γ lengkap (pioglitazone).

HAEMODYNAMIC EFFECTS OF ADIPONECTIN ON PPAR–γ RECEPTORS IN DIABETIC AND NON-DIABETIC WISTAR KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS

ABSTRACT

The prevalence of hypertension and diabetes is mounting with unprecedented degree in both developing and advanced countries. Reduced plasma adiponectin concentrations have been found in hypertension and diabetes. Adiponectin, an adipokine, is a protein hormone which mediates its action by stimulating nitric oxide (NO) release from the vascular endothelium thereby causing vasodilation. PPAR-γ is a member of Peroxisome proliferator activated receptor, which is positive regulator of adiponectin gene expression. In this study irbesartan has been used as partial PPAR-γ agonist, by contrast, pioglitazone acts as full ligand for PPAR-γ, which causes increases in adiponectin plasma concentration. This study investigated the antihypertensive, anti-diabetic, antioxidant potential and renal haemodynamics of adiponectin with PPAR-γ agonists in diabetic and non-diabetic model of rats. Besides, the effect of adiponectin and combined treatment of adiponectin with partial or full PPAR-γ agonists on the α_1-adrenoceptor subtypes responsiveness in systemic and renal vasculature alteration of diabetic and non-diabetic normotensive and hypertensive rats was explored. Type 1 diabetes was induced using a single intra-peritoneal injection of streptozotocin at a dose of 40 mg/kg body weight, dissolved in sodium citrate buffer (pH 4.5). One set of diabetic normotensive (WKY) and hypertensive (SHR) rats received irbesartan, pioglitazone,

adiponectin and combination of adiponectin with either irbesartan or pioglitazone, while the other set of non-diabetic WKY and SHRs received the same pattern of treatment. Blood pressure (B.P) and metabolic parameters were measured in conscious rats. In addition pulse wave velocity, renal cortical blood perfusion, plasma adiponectin, lipid profile and electrolytes were also measured during and at the end of study. Moreover, rats were anaesthetized with sodium pentobarbitone and reductions in mean arterial pressure and renal cortical blood flow induced by systemic and intra-renal administration of noradrenaline, phenylephrine, methoxamine and angiotensin II (Ang II) were determined. Data, mean±SEM were subjected to ANOVA with significance at $P<0.05$. Diabetic SHRs had higher B.P, low plasma adiponectin, renal dysfunction marked by increased plasma creatinine, creatinine clearance, absolute and fractional sodium excretion. Treatment with adiponectin alone and combination of adiponectin with either partial or full PPAR-γ agonists reduced B.P, blunted systemic and renal vascular response to α_1-adrenergic agonists and Ang II, ameliorate oxidative stress and improved renal haemodynamics and excretory functions, thus signify a complex interaction between α_1-adrenoceptor subtypes, Ang II and adiponectin receptors. Moreover, combined adiponectin with pioglitazone underlie a reno-protective role of adiponectin, and signifies a degree of synergism exist between adiponectin and full PPAR-γ agonist (pioglitazone) treatment.

CHAPTER 1: INTRODUCTION

1.1 Kidney

1.1.1 Anatomy of kidney

In human beings, there is a pair of kidneys, one on each side of spine and located in the abdominal cavity called the retroperitoneal space. The exact location of kidneys in human beings is approximately at the vertebral level T12 to L3 (Walter and Boron, 2004). Each adult kidney weighs between 125 and 170 grams in males and between 115 and 155 grams in females. The kidney is approximately 11–14 cm in length, 6 cm wide and 4 cm thick. The left kidney is typically slightly larger than the right (Glodny et al. 2009).

Macroscopically each kidney has been described as a bean-shaped organ having both concave and convex surfaces. On the concave surface i.e., the medial side, there is a depression called hilum, at which the renal artery and nerve enters the organ, whereas the renal vein and ureter leaves the kidney (Marieb and Hoehn, 2007). In a cross section of the kidney, two major regions can be identified. The outer or superficial reddish brown region, which is granular in appearance, called cortex and inner or deep darker brown, appears striated is known as medulla. Nephrons, the urine-producing functional unit of the kidney, span the cortex and medulla (Shier, 2003). There are about 20 million nephrons in each kidney. The initial filtering portion of a nephron is made of glomerulus and Bowmans capsule collectively called renal corpuscle, located in the cortex, which is followed by renal tubule that passes from cortex deep into the medullary pyramids (Walter and Boron, 2004). The renal tubules consist of proximal convoluted tubule, loop of Henle and distal convoluted tubule. Kidneys receive blood from the respective

renal artery, left and right, which branches directly from the abdominal aorta. Each renal artery branches into segmental arteries, dividing further into interlobar arteries that penetrate the renal capsule and extend through the renal columns between the renal pyramids. Each arcuate artery supplies several interlobular arteries that feed into the afferent arterioles that supply the glomeruli (Walter and Boron, 2004). After filtration, the blood moves through a small network of venules that converge into interlobular veins. As with the arteriole distribution, the veins follow the same pattern, the interlobular veins provide blood to arcuate veins then back to interlobar veins that unite to form the renal vein that exits at the hilus of the kidney. Renal veins return the blood to inferior vena cava (Vander, 1995, Applegate, 2000, and Meyer et al. 2004).

The kidney and nervous system communicate via the renal plexus, whose fibres' course along the renal arteries to reach the kidney. Input from the SNS triggers vasoconstriction in the kidney, thereby reducing renal blood flow. Interestingly kidney is devoid of input from the parasympathetic nervous system (Bard et al. 2003).

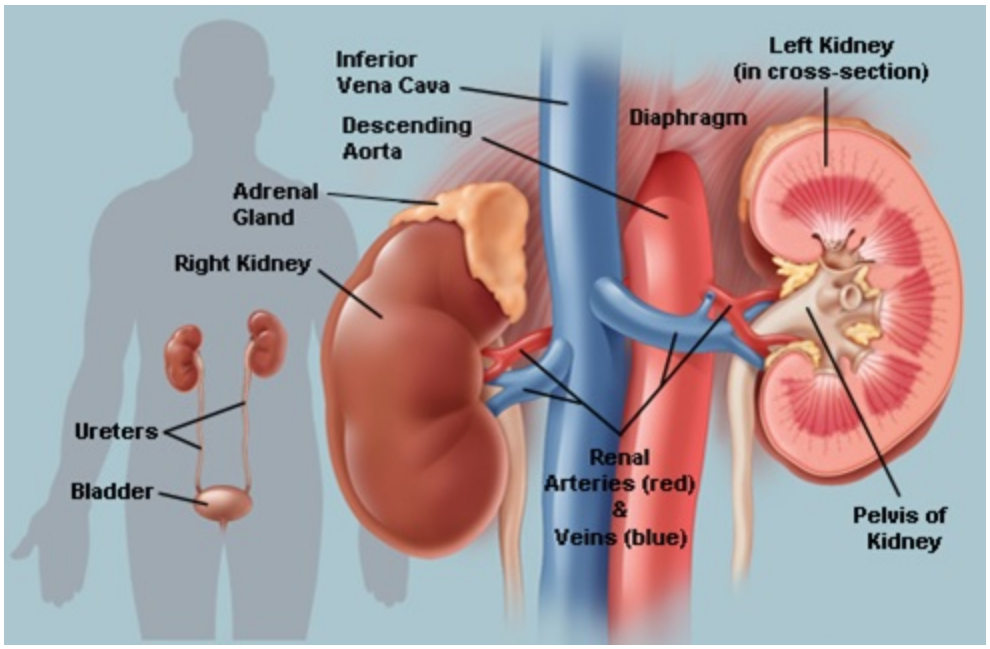

Figure 1.1: Gross anatomy of kidney

(Adapted from 2009 WebMD, LLC, Anatomy of the Human Body).

1.1.2 Physiology of Kidney

Kidneys contribute in whole-body homeostasis through the excretion of waste products of metabolism like urea and uric acid, regulation of acid base balance, electrolyte concentrations and extracellular fluid volume. Kidneys are also involved in the reabsorption of important nutrients like glucose and amino acids. The production of various hormones like erythropoietin and activation of vitamin-D also come under the functions of kidneys (Dantzler, 1989). Most of kidney's functions are completed by the simple mechanisms of filtration, reabsorption and secretion that take place within the nephron. Filtration occurs at the renal corpuscle. It is the process by which cells and large proteins are filtered from the blood to make the glomerular filtrate that eventually becomes urine. The kidney produces about 180 liters of ultra filtrate per day, while

reabsorbing a large percentage, allowing for the production of only approximately 2 liters of urine. Reabsorption is the transport of molecules from this ultra filtrate and into the blood. On the other hand, secretion is the reverse process, in which molecules are transported in the opposite direction, from the blood into the urine (Guyton, 1991b). One of the most vital functions that the normal kidneys perform is the long-term regulation of blood pressure. This regulation occurs through preservation of the extracellular fluid compartment, the size that depends on plasma sodium concentration through the activation of renin angiotensin-aldosterone system (Hall and Guyton, 2011). Kidneys work in conjunction with cardiovascular, endocrine and nervous system in order to maintain the blood pressure (Germann et al. 2005).

1.2 Cardiovascular system

1.2.1 Anatomy of cardiovascular system

The cardiovascular system distributes the blood and consists of the heart, blood and blood vessels. One of the essential components of cardiovascular system is the closed circulatory system that permits blood and lymph circulation in order to transport nutrients and waste products to and from cells in body (Dorland, 2011). In humans, the circulatory system includes the pulmonary circulation and systemic circulation. Pulmonary circulation is a loop through the lungs where blood gets oxygenated, whereas the systemic circulation provides oxygenated blood to the rest of the body (Guyton and Hall, 2006). The deoxygenated blood is brought back to the right atrium via the superior and inferior vena cava and follow through the tricuspid valve to the right ventricle, from where it is pumped through the pulmonary artery to lungs. Gaseous exchange occurs in the lungs and pulmonary vein returns the oxygenated blood to left atrium. Conversely,

systemic circulation transports oxygen rich blood away from the heart to the body except lungs from the left ventricle through aorta and bring back the oxygen-depleted blood back to the heart (Guyton and Hall, 2006).

1.2.2 Heart and blood vessels

The human heart is composed of cardiac muscle, which is an involuntary striated muscle tissue found only in this organ, and connective tissue. On the average human heart, beats 72 times per minute, roughly beats 2.5 billion times during an average sixty-five years of lifespan, and weighs approximately 300 to 350 grams (Kumar et al. 2005). The heart is muscular conical organ that lies between the lungs in the middle mediastinum and is enclosed in the pericardium. Heart is placed obliquely anterior to the vertebral column and behind the body of sternum so that 1/3 of it lies to the right and 2/3 lies to the left of median plane. It is enclosed in a double-walled sac called the pericardium. The superficial part of this sac is called the fibrous pericardium (Gavaghan, 1998). The outer wall of the human heart is composed of three layers. The outer layer is called the epicardium, or visceral pericardium since it is also the inner wall of the pericardium. The middle layer is called the myocardium and is composed of muscle that contracts. The inner layer is called the endocardium and is in contact with the blood that the heart pumps and it merges with the inner lining (endothelium) of blood vessels and covers the heart valves (Iles and Docherty, 2011).

The blood vessels refer to the closed system of tubes that transport blood to all parts of the body and back to the heart. The blood vessels consist of arteries, arterioles, capillaries, venules, and veins. The actual exchange of oxygen, carbon dioxide and waste

matter between the blood and the tissue fluid occurs in microscopically small vessels, called capillaries (Hall, 2010). Structurally the wall of arteries composed of three layers viz; tunica intima, tunica media and tunica adventitia. Tunica intima consists of an inner surface of smooth endothelium covered by a layer of elastic tissues. The tunica media is thicker in arteries and consists of smooth muscle cells mixed with elastic fibers. Tunica media of larger vessels is primarily composed of elastic fibers. Tunica adventitia of blood vessels is composed of collagenous and elastic fibres (Derrickson and Tortora, 2006, Human cardiovascular system, 2013). Progressive thinning of the vessel wall and a decrease in the size of the lumen results in the formation of arterioles that provides the most of the peripheral resistance (Tobian et al. 1961).

1.2.3 Physiology of Cardiovascular system

The heart acts as a functional syncytium and is divided into the right side and left side heart. Function of the right side of heart is to collect de-oxygenated blood, in the right atrium, from the body via superior and inferior vena cava and pump it to right ventricle, through the tricuspid valve into the lungs termed as pulmonary circulation. In the lungs there is oxygenation of blood through the passive process of diffusion. The left sided heart collects oxygenated blood from the lungs into the left atrium. From the left atrium the blood moves to the left ventricle, through the bicuspid valve, which pumps it out to the body via the aorta. On both sides, the lower ventricles are thicker and stronger than the upper atria. The muscle wall surrounding the left ventricle is thicker than the wall surrounding the right ventricle due to the higher force needed to pump the blood through the systemic circulation (Scott, 1986). The one complete beat of the heart that is one systole followed by one diastole is referred as one cardiac cycle. The duration of one

cardiac cycle in human beings is 0.8 seconds. Systole lasts for 0.3 seconds and diastole lasts for 0.5 seconds (Guyton and Hall, 2006). The heart is responsible for pumping blood throughout the blood vessels by repeated, rhythmic contractions and acts as a functional syncytium. Cardiac contractions are managed by specialized and self-excitatory conductive system of the heart. This conducting system consists of sinoatrial node or S-A node, in which the normal rhythmic impulse is generated; internodal pathway; A-V node; A-V bundle and right and left bundles of Purkinje fibers. Automacity is the process that can cause automatic rhythmical contractions and is best expressed in S-A node that is also known as pacemaker of the heart. Once a cardiac impulse is generated at the S-A node, it subsequently spread to all parts of heart through the conducting system resulting in rhythmic contractions and ventricles provide the major source of power for moving blood in the vascular system (Hall & Guyton, 2011). The cardiovascular system serve the needs of the tissue including transport of nutrients to tissue, transport of waste products away from tissues, hormones transport , maintenance of pH, thermoregulation, providing the necessary cardiac output and arterial pressure, preservation of fluid balance and to maintain homeostasis. The cardiovascular system works in concurrence with other body systems such as nervous, endocrine, renal and respiratory to maintain a suitable and steady environment.

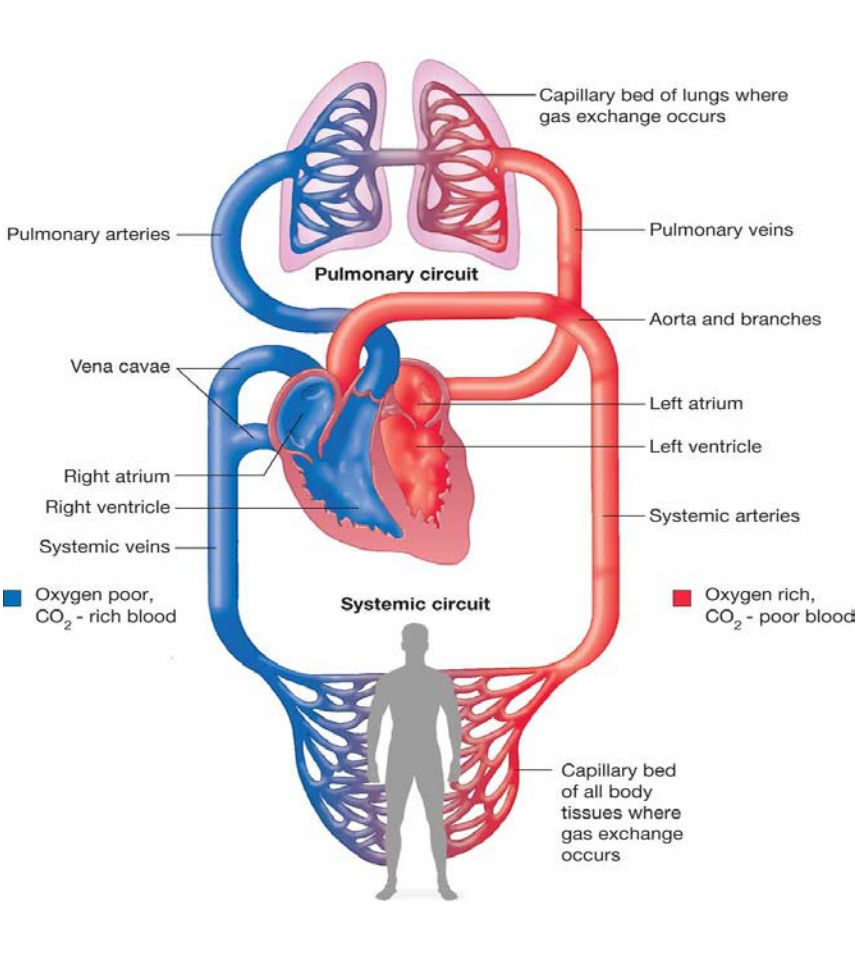

Figure 1.2 Gross anatomy of cardiovascular system of heart (Adapted from Pearson Education, Inc. Publishing, 2013).

1.3 Hypertension

Hypertension is a common worldwide health problem and about 40% of individuals had been diagnosed with this chronic illness in year 2008 (World Health, 2013). According to the Joint National Committee 7 (JNC 7), hypertension is defined as physician office systolic BP level of ≥ 140 mmHg and diastolic BP of ≥ 90 mmHg. The gray area between systolic BP of 120-139 mmHg and diastolic BP of 80-89 mmHg is defined as "prehypertension" (Chobanian et al. 2003).

1.3.1 Blood Pressure

Blood pressure is defined as the pressure exerted by the blood against any unit area of vessel wall (Guyton, 1991a). Blood pressure is the resultant of the activity of heart and blood vessels. Simply, blood pressure is equal to cardiac output multiplied by total peripheral resistance. The normal blood pressure ranges are systolic blood pressure 90-119 mmHg and diastolic blood pressure 60-79 mmHg (Chobanian et al. 2003). Many interrelated physiological mechanisms are involved in maintaining the blood pressure including sympathetic and parasympathetic nerves, baroreceptors, circulatory hormones and local auto-regulatory mechanisms. Derangement in these factors contributes to the elevation of blood pressure (Beevers et al. 2001).

Table 1.1: Classification of blood pressure

Classification	Systolic BP (mmHg)	Diastolic BP (mmHg)
Normal	**<120**	**And <80**
Pre-hypertension	**120-139**	**Or 80-89**
Stage 1 hypertension	**140-159**	**Or 90-99**
Stage 2 hypertension	**≥ 160**	**≥ 100**

BP = blood pressure (Human cardiovascular system), Data from National heart, lung and blood. Institute: www. britannica.com/ hypertension (accessed November, 2013).

1.3.2 Primary and secondary hypertension

Hypertension is divided into primary or essential and secondary hypertension. Primary hypertension is where there is rise of blood pressure due to unknown cause with a subsequent increased risk of cerebral, cardiac and renal complications (Messerli et al., 2007). Essential hypertension accounts for 90-95% cases of hypertension. The term secondary hypertension refers to remaining 5-10% cases of hypertension of known

origin. Primary hypertension, where there is no identifiable cause, is often a result of complex interactions between multiple environmental and genetic factors. On the other hand, identifiable causes of secondary hypertension include genetic syndromes, renal disease, renal vascular hypertension, Cushing syndrome, primary hyperaldosteronism, pregnancy, pheochromocytoma, hypercalcemia and medications (McPhee et al. 2010).

1.3.3 Pathophysiology of hypertension

Many pathophysiological mechanisms are involved in the genesis and maintenance of hypertension. However, extensive experimental and clinical data supports the view that impaired renal functions play a primary and vital role in the pathogenesis of hypertension as proposed by Guyton and Hall (Hall and Guyton, 2011). Pathophysiology is quite complex and multi-factorial, all these factors interact with each other through complex mechanisms that results in an increase of blood pressure, and related target organs damage (Feinleib et al. 1977, Longini et al. 1984). However, it has been suggested that the genetic causes of hypertension is uncommon in general hypertensive population. Moreover, the genetic predisposition can be expressed fully by the interaction of environmental and demographic factors (Oparil et al. 2003). Likewise, hypertension and type II diabetes mellitus coexist (Haffner et al. 1998). Similarly, metabolic syndrome also has been proposed in the pathogenesis of hypertension (Epstein et al. 1996). It has been proposed that over activity of SNS contributes to the development and maintenance of hypertension through increased cardiac output, increased vascular resistance and abnormal water retention (Mark, 1996). It has been reported that the vascular reactivity of hypertensive patients is greater as they exhibit greater vasoconstrictor responses to infused noradrenaline than normotensive persons (Ziegler et al. 1991).

Renal vasoconstriction, renal ischemia, local generation of reactive oxygen species and over production and activation of local angiotensin II act as stimului for renal vasculopathy and hemodynamic effects leading to hypertension (Sealey et al. 1988). Vascular remodeling resulting in an increased vascular peripheral resistance is another feature of hypertension. Such that alteration in structure and functions of small arteries contribute to the elevation of blood pressure (Mulvany and Aalkjær, 1990). Since the discovery of renin-angiotensin-aldosterone system (RAAS), its role in the pathogenesis of hypertension had been extensively studied and was found to be the key factor in the genesis and maintenance of hypertension. RAAS contributes in the elevation of blood pressure through multiple mechanisms including vasoconstriction of resistance vessels, release of aldosterone, renal sodium reabsorption, release of anti-diuretic hormone and augmenting of central sympathetic outflow. Angiotensin II (Ang II) has also been implicated in cardiac and vascular cell hypertrophy and hyperplasia through the activation of angiotensin type I receptors and by stimulating the release of growth factors and cytokines (McConnaughey et al. 1999). Similarly, aldosterone excess has been now considered as common contributing factor in hypertension (Lijnen and Petrov, 2000). Moreover, arterial stiffness and high levels of circulatory endothelin have also been proposed in the development of hypertension (Ergul et al. 1996).

The concept of oxidative stress and endothelial dysfunction contributing to the pathogenesis of hypertension has gained interest in recent years. Angiotensin II-induced activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase results in an increased production of oxidant superoxide anions ($O2^{\bullet -}$). Increased $O2^{\bullet -}$ production results in oxidative inactivation of nitric oxide (NO) and the resultant decreased bio-

availability of NO provides another mechanism of increased vasoconstriction and hypertension (Rajagopalan et al. 1996). Adipose tissue releases leptin, angiotensinogen and oxidized fatty acids to stimulate adrenal release of aldosterone via activation of the classic RAAS, as well as a non-classical pathway mediated by oxidized fatty acids. Leptin stimulates the central SNS, which in turn leads to renin release from the kidney. Activation of RAAS in other tissues contributes to renal and vascular dysfunction. Increased adipose tissue can lead to obstructive sleep apnoea (OSA), which can be treated by therapeutic weight loss or application of continuous positive airway pressure (cPAP). OSA leads to activation of the sympathetic nervous system (SNS), which activates RAAS in the kidney. Increased aldosterone can be reduced with mineralocorticoid receptor antagonists, (Figure 1.3).

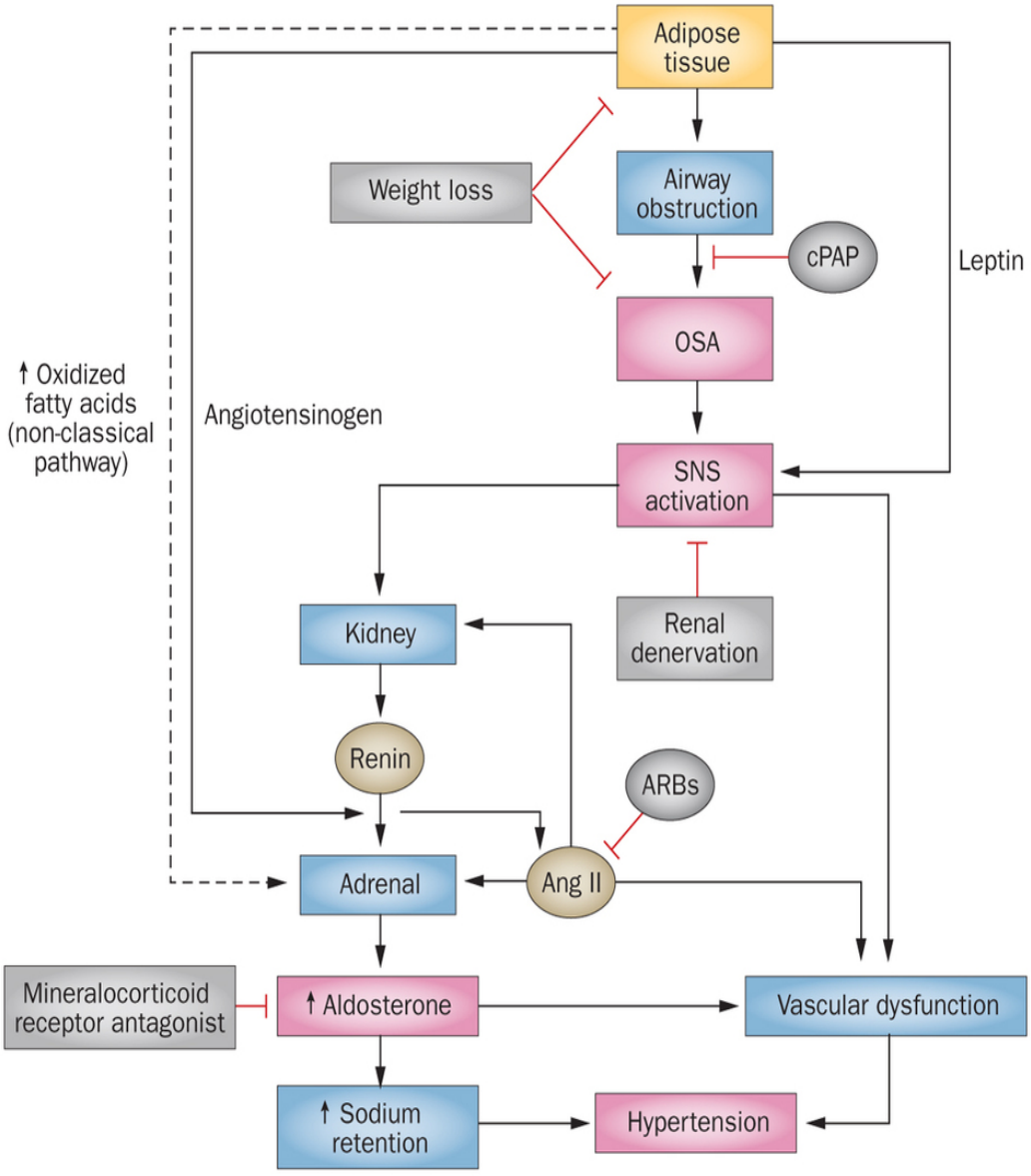

Figure 1.3: Pathophysiological mechanisms of hypertension

(Adapted from DeMarco et al. 2014).

Cpap: continuous positive airway pressure; **OSA**: obstructive sleep apnoea, **RAAS**: renin–angiotensin–aldosterone system; **SNS**: sympathetic nervous system, **ARB:** angiotensin receptor blocker, **Ang II:** angiotensin II.

1.4 Diabetes

There has been an increase in the prevalence of diabetes mellitus over the past 40 years, both in the US and worldwide. The world wide prevalence of diabetes in year 2000 was approximately 2.8% and is estimated to grow to 4.4% by the year 2030, that will lead to a rise in the number of diabetic patients from 171 million in year 2000 to 350 million in year 2030 (Wild et al. 2004).

Diabetes mellitus is one of the most important public health problems prevailing worldwide with a leading cause of death and huge economic burden. Until the early part of the 20th century, being diagnosed with diabetes used to mean certain death within few years due to the lack of proper therapeutic options. The revolutionary discovery of insulin allowed a control of this disease in terms of reduced mortality and morbidity. The advancement of science also enabled to undertake in-depth studies regarding the related intricate pathophysiology of diabetes and brought the adverse effect of this disease in forefront of medical and health research. The increasing prevalence and globalization of this disease has increased and is particularly observed in the recent decades (Kennedy and Zochodne, 2005).

Diabetes is defined as a chronic metabolic disorder that affects the metabolism of carbohydrates and other nutrients because of impaired insulin release and/or insulin resistance resulting in hyperglycemia (Tierney et al. 1996) Diabetes mellitus is classified into insulin dependent diabetes mellitus (IDDM) or type 1 diabetes and non-insulin dependent diabetes mellitus (NIDDM) or type 2 diabetes. The type 1 diabetes is mainly related to insulin deficiency in which a sequel of β-cell destruction is widely implicated

in the pathogenesis of this type of diabetes. The type 2 diabetes may be caused due to either cellular insensitivity to insulin or insulin resistance and/or secretory dysfunction related to impaired β-cell function or the presence of non-functional β-cells. Type 1 diabetes mellitus which is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to a deficiency of insulin. It is either immune-mediated or idiopathic (Boon & Davidson, 2006). The majority of type 1 diabetes is of the immune-mediated in nature, where the loss of beta cells is due to autoimmune attack mediated by T-cell (Johns Hopkins Autoimmune Disease Research Center, 2007). On the other hand, Type II diabetes mellitus is characterized by the presence of insulin resistance or reduced insulin sensitivity, and with relatively reduced insulin secretion. This defective response of body tissues to circulating insulin involves insulin receptor in cell membranes (Rother, 2007). The exact cause and mechanism is not fully understood in type II diabetes mellitus. Certain risk factors are associated with increased incidence of diabetes such as obesity, as 55% of patient diagnosed with type 2 diabetes mellitus have central obesity. Other factors include ageing, increasing body mass and decrease demands of physical activity and family history (Kopelman, 2000). Genetics are strongly linked with both types of diabetes mellitus (Walley et al. 2006). The hyperglycemia due to poor glycemic control is common in overt diabetes and is associated with dysfunctions of different organs, particularly kidney, nerves, eye, heart and blood vessels. (Cooper et al. 2001).

1.4.1 Diabetes and hypertension

Hypertension and diabetes often coexist. Diabetics have increased prevalence of developing the hypertension. One prospective study (that included 12,550 adults),

indicates that the development of diabetes in hypertensive patients is 2.5 times greater as compared to normotensive subjects (National High Blood Pressure Education Program Working Group, 1994). Similarly, previous data suggests that there is increased prevalence of diabetes in hypertension and approximately 20% of hypertensives have coexisting diabetes (Contreras et al. 2000). Moreover, both diseases serve to induce and as well as exacerbate each other (Sowers and Epstein, 1995). Both hypertension and diabetes predispose to the development of cardiovascular disease (CVD) and renal disease as their major complications (Sowers, 2004). The co-existence of hypertension and diabetes in patients increases the risks of cardiovascular diseases by 75% (Adler et al. 2000). Diabetes mellitus & systemic hypertension promote the process of atherosclerosis, and their combination further increases this risk (Fuller, 1985).

1.4.2 Complications of diabetes in hypertension and oxidative stress

Hypertension and diabetes are associated with marked abnormalities of cardiovascular structure and functions. Hypertension and diabetes both can induce coronary heart disease, infarction, cerebrovascular accidents, nephropathy and retinopathy and peripheral vascular diseases (Bakris et al. 2000). Diabetes is commonly associated with both micro- and macro-vascular complications. These vascular complications are mainly accelerated in context of systemic hypertension. The underlying molecular mechanisms responsible for diabetic vascular complications are being elucidated. A large body of research is examining this topic and it appears that in case of diabetes, both metabolic and hemodynamic factors interact to stimulate the expression of cytokines and growth factors in the various vascular trees and contribute in the genesis of these complications.

Diabetes provides a distinct model of chronic vascular disease in which altered glycemic status of the body results in multiple organ dysfunctions. These diabetes-induced complications can divided into micro- and macro-vascular complications. Neuropathy, nephropathy and retinopathy are major diabetic micro-vascular complications of diabetes. The macro-vascular complication of diabetes is manifested as accelerated atherosclerosis that predisposes the patients to premature ischemic heart disease, increased risk for cerebrovascular disease and for severe peripheral vascular diseases. Several factors including metabolic, humoral & hemodynamic factors are believed to be involved in the pathogenesis of vasculopathy frequently observed in overt and poorly controlled diabetes (Wood et al. 1995).

Hypertension is a chronic medical condition and frequently remains asymptomatic until late in its course. It can be classified as essential (primary) and secondary hypertension. Essential hypertension is of unknown cause and constitutes about 90 to 95% of cases. Many pathophysiological factors contribute to the genesis of essential hypertension such as high sodium intake, inadequate dietary intake of potassium and calcium, increased secretion of renin, angiotensin II and aldosterone, overproduction of sodium-retaining hormones and vasoconstrictors, increased sympathetic activity and deficiencies of vasodilators (Wood et al. 1995).

In hypertension, production of cardiac and vascular ROS is increased. Increase in vascular oxidative stress has been observed in different models of experimental hypertension like angiotensin-II induced hypertension, Dahl salt-sensitive hypertension, obesity-associated hypertension, and SHR. Antioxidants and SOD mimetic decreased blood pressure and prevented the development of hypertension in animal models of

hypertension (Park et al. 2002). These beneficial effects point towards the role of ROS in the development of hypertension and vascular complications. In hypertension, antioxidants may improve endothelial function, regress vascular remodeling and reduce vascular inflammation.

Endothelial dysfunction is considered as the first step in the pathogenesis of micro and macro vascular complications of both diseases. Endothelium, the inner lining of blood vessels, releases certain chemical substances in response to acetylcholine. These substances are divided into two types according to the functions they perform (Wong et al. 2010). Endothelium-derived relaxing factors (EDRFs) including nitric oxide (NO), prostacyclin (PGI2) and endothelium-derived hyperpolarizing factors (EDHFs). All of them reduce the vascular tone. Opposite to the beneficial EDRFs endothelium also produces a vasoconstrictors substance called as endothelial-derived constrictors factors (EDCFs). Prostaglandin H2, thromboxane A2 (TXA2), leukotrienes, endothelin, and superoxide anions are included in this category (Vanhoutte, 2009). A critical balance is required between EDRFs and EDCFs in order to maintain the vascular health and function. Hypertension and diabetes tend to disturb this balance via either increasing or decreasing the production of one or both (Vanhoutte et al. 2009). Hyperglycemia associated with diabetes modifies the endothelial function through a numbers of complex mechanisms including oxidative stress (Laight et al. 2000), glycation of protein and lipids (Vlassara et al. 1992), and activation of protein kinase C (Hink et al. 2001). Similarly, the endothelial dependent vasodilatation is impaired in different animal models of hypertension including spontaneous hypertensive rats and renovascular hypertension (Quaschning et al. 2006). ROS formation can be a direct consequence of hyperglycemia.

Hyperglycemia induced endothelial cell mitochondrial overproduction of superoxide is involved in the pathogenesis of diabetes related complications. It has been proven that the inhibition of hyperglycemia induced overproduction of superoxide by manganese superoxide dismutase completely prevents advanced glycation end-product (AGE) formation, protein kinasae C activation (PKC) and the hexosamine pathway in endothelial cells (Brownlee, 2001, Nishikawa et al. 2000). Chronic hyperglycemia enhances the local activity of renin-angiotensin-aldosterone system (Nickenig et al. 1998), which leads to development of wall hypertrophy and fibrosis. Hyperglycemia can induce superoxide and lower SOD activity leading to impaired endothelium-dependent vasodilatation in diabetes (Du et al. 2003). Hyperglycemia is thought to increase the production of Ang II in various tissues, including blood vessel wall, kidney and heart. Ang II stimulates NAD(P)H oxidase via the AT1 receptor, leading to an increase in the tissue formation of reactive oxygen species (O2, OH and H2O2). Reactive oxygen species induce endothelial dysfunction reducing bioavailability of nitric oxide. Endothelial dysfunction leads to the release of endothelin and catecholamines that induce vasoconstriction (Giacchetti et al. 2005).

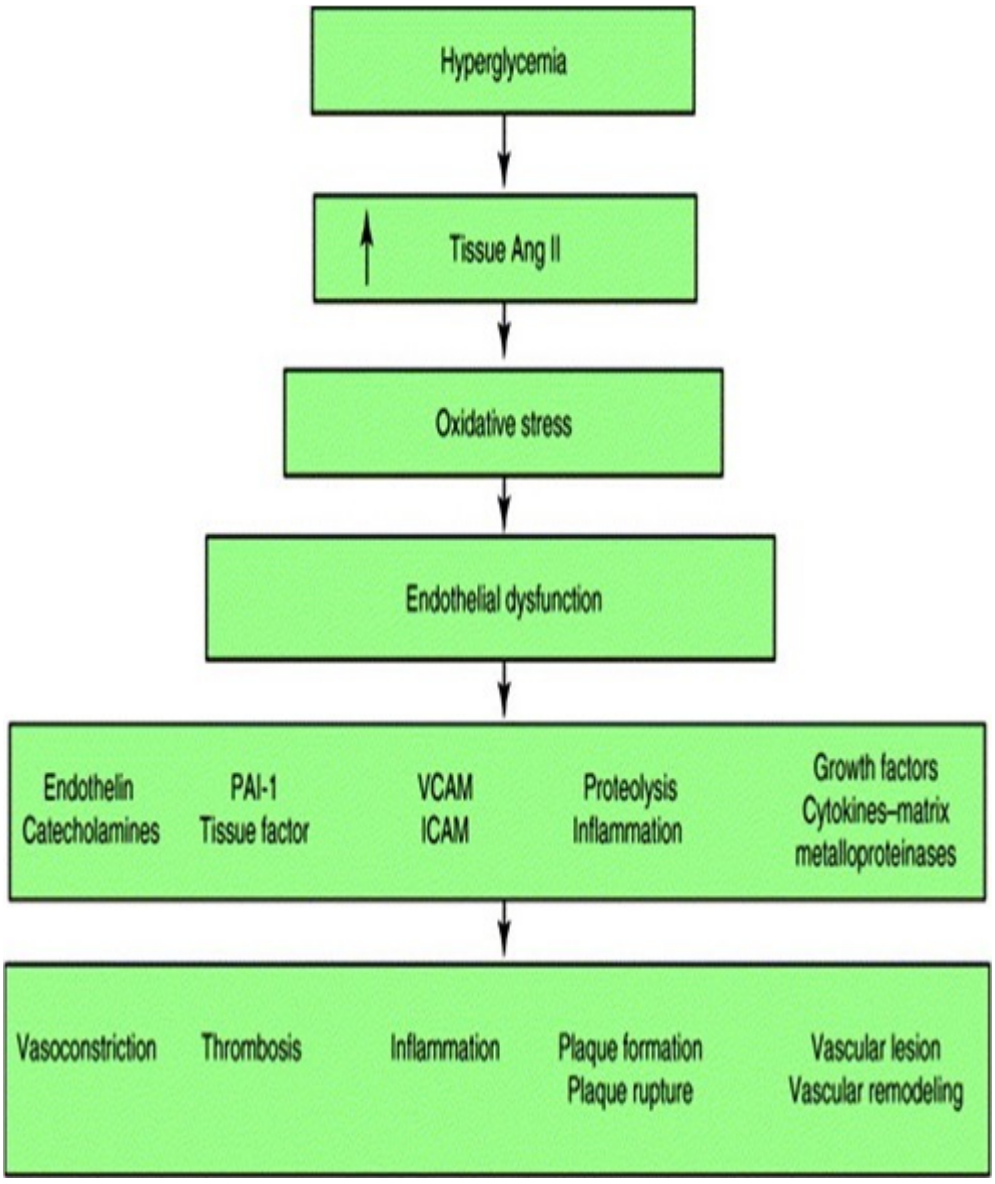

Figure1.4: Schematic representation of the involvement of oxidative stress induced by diabetes and the development of diabetic complications

(Adapted from Giacchetti et al. 2005)

PAI-1: plasminogen activator inhibitor-1, **VCAM:** vascular cell adhesion molecule, **ICAM:** intercellular adhesion molecule