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Endothelial dysfunction complications and current status

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Abstract
In past, endothelium was thought to be only a mechanical barrier. In present time, endothelium is known to be a tissue regulating vascular tone, cell growth and the interaction between leukocytes, thrombocytes and various blood vessel wall. It also synthesizes growth factors and thrombo-regulatory molecules and responds to physical and chemical stimuli. Even though endothelial dysfunction is a complex term in itself. It is generally used for the incompetent endothelium-dependent vasodilatation, the term also covers the abnormalities between endothelium and thrombocytes, leukocytes and other regulatory protein molecules. Fit and healthy endothelium is considered very essential for cardiovascular control. Thus, endothelium layers play a dynamic role in pathogenesis of many diseases and cardiovascular problems such as congestive heart failure, atherosclerosis, systemic and pulmonary hypertension, cardiomyopathies and vasculitis. The main aim of this article is to enlighten the endothelial dysfunction and the circulating molecules of endothelial cells as they become potential targets of therapeutic approach for hypertension and other cardiovascular diseases. This article also reviews the role and importance of endothelial dysfunction in hypertension by addressing the nature of the endothelial function, different mechanisms of the endothelial dysfunction and its relationship with the disease. The key associations between hypertension and endothelial dysfunction are vitally very important for future studies to allow new and novel interventions to be designed and released.

Keywords: Endothelial dysfunction, hypertension, fibrinolysis, inflammatory mediators, endothelium-derived relaxing factors, endothelium-derived contracting factors, pathophysiology

1. Introduction
The endothelium is a thin membrane that outlines the inside of the heart and blood vessels. Endothelium comprises of cells that coat the interior surface of the blood vessels and lymphatic vessels [1]. Making a boundary between circulating blood or lymph in the lumen and the rest sections of the vessel wall. Endothelial are the cells that release substances which control the vascular contraction and relaxation as well as enzymes that control blood clotting, immune function and platelet (A colourless substance in the blood) adhesion [2]. Vascular endothelial cells are those which are in direct contact with the blood, whereas those in direct contact with lymph are known as the lymphatic endothelial cells [3].
Endothelium is mesodermal in origin. Both lymphatic capillaries and blood are composed of a single layer of endothelial cells called a monolayer. In straight sections of a blood vessel, vascular endothelial cells typically align and extend in the direction of fluid flow [4, 5].
Multiprotein complexes containing transmembrane proteins (such as claudins, occludins, and junction adhesion molecules) and cytosol proteins that connect membrane proteins to the intracellular cytoskeleton form intercellular junctions between ECs [6]. The endothelium also serves as an endocrine organ, while it demonstrates several paracrine functions by producing and secreting vasoactive, inflammatory, vasculoprotective, angiogenic, thrombotic and antithrombotic molecules (Fig. 1). Like the other endocrine organs, endothelium possesses receptors that display various cellular and hormonal events [7, 8].

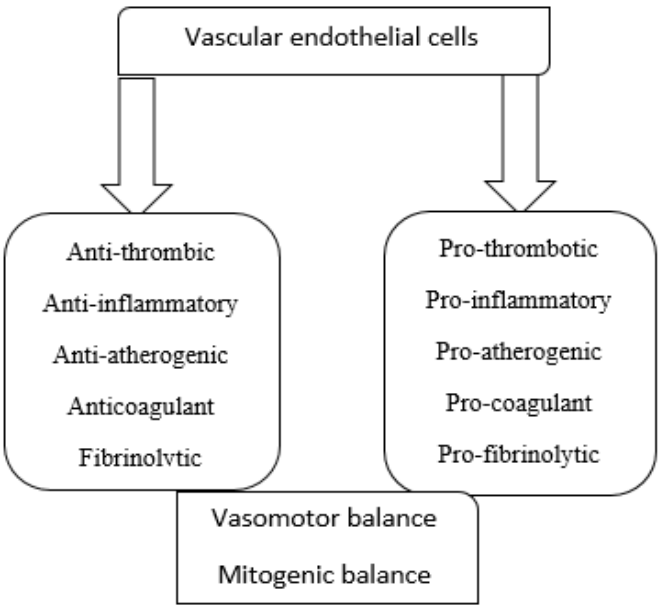

Fig 1: Activities of endothelial cells

1.2 Nature of endothelial function

Three layers of the artery wall from inside to outside comprise; tunica intima, tunica media and tunica adventitia. The layer of tunica adventitia; contains nerve endings, perivascular adipose tissue and connective elements, such as fibroblasts and collagen. It plays important roles in the vascular development and remodelling [9, 10]. The second layer, vascular smooth muscle, regulates the response of constriction and dilatation of the blood vessels. The mechanical stimuli, such as shear stress and pressure, or pharmacological stimuli activate the contraction of the vascular smooth muscle cells by increasing the intracellular calcium concentration. Tunica intima which is the innermost layer of the vascular arterial wall, consists of monolayered endothelial cells and connective tissues lie beneath the ECs [11, 12]. Substances can pass through the joining between the endothelial cells or are absorbed by the endothelial cells. As the vascular vessel sizes are about 60–80 nm in diameter, endothelium provides restriction for larger particles and prevents the interaction between the blood cells and the vessel wall [13, 14].

1.3 Nitric Oxide

Vascular smooth muscle cells release most powerful vasodilator NO which activates the soluble guanylate cyclase. Soluble Guanylate cyclase (sGS) enzyme converts GTP to cyclic GMP (cGMP) which then activates protein kinase C that causes decrease in the cytosolic calcium concentrations of the cells. NO can also affect cellular activity, independently of sGC activation, by the stimulation of the endoplasmic reticulum calcium ATPase, reducing the intracellular calcium concentration and cause relaxation of the smooth muscle. The release of inflammation, vascular cell proliferation, platelet adhesion, and tissue factors are inhibited by NO [15].

NO is produced from an L-arginine by the enzyme nitric oxide synthases (NOS) as a free radical (Fig. 2). There are three distinct genes encoding NOS isozymes; neuronal NOS (nNOS or NOS-1), cytokine-inducible NOS (iNOS or NOS-2) and endothelial NOS (eNOS or NOS) [16]. The production of NO from L-arginine by NOS involves the presence of various co-factors including tetrahydrobiopterin, flavin mononucleotide, flavin adenine dinucleotide, calmodulin (calcium binding proteins) and iron protoporphyrin [17]. nNOS is expressed in the central and peripheral nervous systems, in cardiac and skeletal myocytes, smooth muscles and ECs. NO produced in the nervous systems by nNOS is associated with the regulation of neuronal excitability and synaptic plasticity, memory and learning processes. It has been suggested that the expression of vascular nNOS is also upregulated by stimulation with AT-II and platelet-derived growth factor. When iNOS is stimulated, it continuously produces NO. Induction of iNOS arises mainly during chronic inflammation and infection. It is stated that inflammation induced iNOS production in the endothelium is somehow related to the vascular dysfunction by limiting the availability of BH4 proteins for e NOS [18]. eNOS is the major isoform for the regulation of vascular function. The activity of eNOS and the production of NO can be stimulated by shear stress, acetylcholine, bradykinin and histamine by both calcium-dependent and independent ways. The endogenous competitive inhibitor for eNOS is called asymmetric dimethyl arginine (ADMA). The inhibition of eNOS is associated with plasma ADMA levels, and plasma ADMA levels are inversely related to endothelium dependent vasodilation. The chronic and acute rise in the shear stress of blood up-controls the activity and expression of eNOS, and thus the release of EDRF/NO. AT-II by binding to its receptor produces bradykinins which stimulate eNOS consequently increases the formation of NO [19].

1.4 Inflammatory and immune response of endothelial cells (EC)

Many stimuli associated with inflammatory and immune vascular diseases have been reported to induce endothelial cell apoptosis [20]. Endothelial cells produce and react to a variety of cytokines (these include chemokines, colony-stimulating factors (CSF), Interleukins (IL), growth factors, and interferons (IFN) and other mediators). Therefore, ECs have important roles in defence and inflammation. The chemokines from ECs affect leukocytes (neutrophils, eosinophils), T lymphocytes, natural killer cells and monocytes. Since endothelial cells are located at the tissue-blood interface, they present several chemokines to the circulating leukocytes. When production of chemokines is elevated, Tumour Necrosis Factor (TNF)-α and IL-1 for the receptor (so called as decoy receptor) are released into the circulation [21]. IL-1 and TNF-α are synergistically D. Konukoglu and H. Uzun effective on the expression of pro-inflammatory genes in various cells. Endothelial cells also produce granulocyte macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF), the stem cell factors, IL-1 and IL-6 and TNF receptors. ECs by themselves are targets of the inflammatory response. TNF-α and TNF-β are produced by activated macrophages and activated T cells, respectively. These trigger endothelial cells and neutrophil aggregation, as well as NO synthesis. Inflammatory disease development depends on the balance between pro-inflammatory and anti-inflammatory cytokines. ECs involve the systemic anti-inflammatory response by producing anti-inflammatory cytokines such as an IL-1 receptor, IL-10, IL-13, and Transforming Growth Factor (TGF)-β. Anti-inflammatory cytokines can either block the process initiated by pro-inflammatory cytokines or suppress the inflammatory cascade. While cytokines such as IL-4, IL-10, IL-13, and TGF-β suppress the production of IL-1, TNF-

α, other pro-inflammatory cytokines block the production of these cytokines [22]. TGF-β is also produced by macrophages, T cells, and endothelium and generally works as a growth inhibitor of ECs. Additionally, IL-8 stimulates proliferation and migration of ECs and have angiogenic properties [23]. ECs facilitate leukocyte movement into tissues through adhesion molecules such as E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule (VCAM). Resting ECs are considered not to be adhesive to circulating leukocytes. ICAM-2 is expressed on resting ECs, whereas ICAM-1 and VCAM are minimal on resting state and their expression can be increased by cytokines and endotoxin activation. Lymphocytes, platelets, and other leukocytes can interact with ECs under basal conditions via the L-selectin receptor. When lymphocytes are activated, they express integrins, which interact with ICAM and VCAM. L-selectin, as an adhesion molecule, and β2 integrin are involved in the adherence of leukocytes to ECs. Activated ECs also secrete platelet activating factor (PAF) and stimulate the expression of P-selectin and E-selectin. PAF upregulates integrins on leukocytes. Activated platelets binds to CD40 on ECs [24, 25]. The endothelium is also capable of expressing various growth factors including G-CSF, M-CSF, GM-CSF, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and fibroblast growth factors (FGF). CSFs and growth factors produced by the endothelium are also important for haematopoiesis which increases the number of immune cells in the circulation during inflammation [26]. The immune system has important roles in the defence mechanism against infections or in response to tissue injury. Dendritic cells (DC), macrophages, natural killer (NK) T cells, and Toll-like receptors (TLRs) are components of the immune system. ECs actively participate in both innate and adaptive immune responses through producing cytokines and chemokines which recruit phagocytes to the site of infection. Endothelial permeability is also increased, allowing for additional trafficking of immune cells during inflammation. Although ECs at rest do not interact with leukocytes, activated ECs increase the expression of adhesion molecules and chemokines and interact with immune cells during the inflammatory process. ECs also can serve as antigen presenting cells by expressing both MHC I and II molecules and presenting endothelial antigens to T cells during inflammation [27, 28]. Both TLRs (TLR2 and TLR4) and NLRs are expressed in inflamed endothelium. When inflammation is dominated by TH1 cells, ECs express chemokine ligand 10 (CXCL10) and E-selectin, which favours the recruitment of TH1 cells. EC surface molecules such as lymphocyte function-associated antigen (LFA)-3 and ICAM1 increase the production of IL-2 and IL-4 by T cells. ECs with activated T cells enhance IFN-γ production via OX40 (CD134) signalling (OX40 is a member of the TNFR/TNF superfamily and are expressed on the activated CD4 and CD8 T cells). An anti-angiogenic cytokine derived Endothelial Dysfunction and Hypertension from ECs, vascular endothelial growth inhibitor functions to suppress ECs proliferation in a cell cycle-dependent manner lipopolysaccharide, which induce ECs to produce IL-1, IL-8, and monocyte chemotactic protein-1 (MCP-1) [29, 30]. Like LPS, tumour necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) can induce TLR2 expression via an NF-kB-dependent pathway. ECs also express CD14, a known receptor for LPS and IFN-α, which is an important cytokine in regulating innate immune responses against viruses [31, 32]. Under normal healthy conditions, ECs express the lectin-like oxidized low-density lipoprotein (oxLDL) receptors (LOX-1) at low levels. Expression of LOX-1 in ECs is elevated in response to stimulation by oxLDL, pro-inflammatory cytokines, and pro-atherogenic factors such as AT-II. OxLDL also induces cell surface adhesion molecule expression and impair NO production in the endothelial cells by increasing superoxide generation. LOX-1 has a role in the mediation of endothelial phagocytosis of aged red blood cells and apoptotic cells. LOX-1-mediated phagocytotic activity can be inhibited by oxLDL. Thus, LOX-1 is important in endothelial-mediated vascular homeostasis and coagulation prevention under physiological conditions [33, 34]. Recently, it has been shown that ECs also induce cellular signalling by endothelial microparticles (EMPs). EMPs are small plasma membrane-derived vesicles (0.1–1.5 μm in diameter), are released by various cell types during cell activation or apoptosis (a type of programmed cell death). Microparticle formation induced by various factors, including TNF-α, IL-1β, thrombin, calcium ionophore, and reactive oxygen species. Microparticles express surface antigens from their cells of origin which allow for the identification of their sources. Circulating EMPs are biomarkers of inflammation and contribute to the pathological state. Depending on the nature of the stimulus, EMPs contain endothelial proteins such as ICAM-1, integrin, and cadherin. EMPs also have endothelial nuclear materials such as microRNA, RNA, and DNA, which can induce intracellular signalling via the transfer of these nuclear materials and proteins to target cells. EMPs also have pro-coagulant and pro-adhesive properties, which promote coagulation and vascular inflammation. EMPs were also found to bring the maturation of plasmacytoid dendritic cells. Plasmacytoid dendritic cells matured by EMPs secrete pro-inflammatory cytokines IL-6 and IL-8 [35-37].

2. Endothelial dysfunction

The term endothelial dysfunction is commonly used to describe the abnormal metabolism of nitric oxide (NO) or improper balance of several endothelium-derived relaxing as well as constrictor factors. Between the blood and the vascular wall, the endothelium forms both mechanical and biological barrier [38]. Interactions between platelets and leukocytes with the vessel wall, impairment of vascular tone, inflammation, free radical formation and oxidation of lipids and vascular smooth muscle cell proliferation can be activate endothelial cells (ECs) [39].

ECs function by secreting relaxing and/or contracting molecules. ECs are exposed to the shear stress resulting from blood flow and can change mechanical stimuli into biochemical signals or intracellular signals (e.g., proliferation, apoptosis, migration, permeability, and remodelling and gene expression) [40]. As a result, endothelial dysfunction is related to several diseases including atherosclerosis, cancer metastasis, inflammatory diseases and hypertension [41].

Healthy endothelium has some athero-protective role including promotion of vasodilation, antioxidant and anti-inflammatory effects, inhibition of both leukocyte adhesion and migration and smooth muscle cell proliferation and migration. Healthy endothelium has anticoagulant and profibrinolytic effects, as well as the inhibitory effects on platelet aggregation and adhesion. Impaired endothelium dependent vasodilation is also associated with the state of endothelial activation which is characterized by elevated pro-inflammatory and pro-coagulator events (Fig. 2). The major

factors for endothelial dysfunction are a reduction of the NO bioavailability, impairment in the response of vascular smooth muscle to the vasodilators, the elevated sensitivity of ECs against oxidative stress induced endothelial dysfunction and Hypertension vasoconstrictors, increased production of the vasoconstrictor substances, or elevated shear stress. Traditional and non-traditional risk factors for cardiovascular events, diabetes mellitus, atherosclerosis and hypertension are associated with enhanced ROS or increased oxidative stress. Increased oxidative stress is considered as a major mechanism involved in the pathogenesis of endothelial dysfunction. Disturbance of NO metabolism may be due to the elevation in oxidative stress [42].

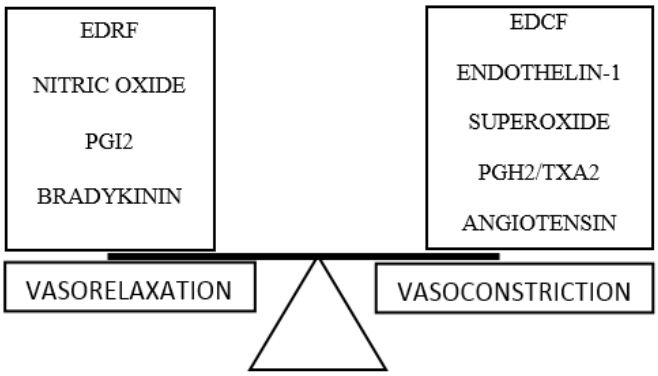

Fig 2: A balance between endothelium-derived relaxing factors (EDRFs) and endothelium-derived contracting factors (EDCFs)

3. Endothelial dysfunction and hypertension

The first report of endothelial dysfunction in human hypertension in the forearm vasculature was reported in 1990 [43]. Impaired vasodilation in hypertension has been long-established by various studies in different vascular endothelial beds including small resistance blood vessels [44]. In stage I essential hypertension, we have shown that ~60% of patients exhibit impaired small artery vasodilation when this is studied *in vitro* on vessels dissected from gluteal subcutaneous biopsies [45]. Impairment of vasodilation has also been described in type 1 and type 2 diabetes, coronary artery disease (12), congestive heart failure, and chronic renal failure [46]. Moreover, this index of endothelial dysfunction not only is associated with cardiovascular disease but may also precede its development, as revealed in a study of offspring of hypertensive patients. The study subjects exhibited endothelial dysfunction despite being normotensive.

An abnormal endothelial function has been drawn in in the pathophysiology of essential hypertension [47]. Endothelial cells produce some of the potent and vital vasoactive substances such as the vasodilator molecules nitric oxide and the vasoconstrictor peptide endothelin-1. Reduction of nitric oxide synthesis or its amplified inactivation by oxygen free radicals may result in increased vascular résistance in hypertensive patients and contribute to clinical consequences of this condition such as vascular and cardiac hypertrophy and brain stroke. The mechanisms triggering impaired nitric oxide availability in hypertension are certainly due to many factors, however, there is significant evidence that nitric oxide bioavailability is reduced because of oxidative inactivation by the undue production of the superoxide anions. In the vascular endothelial wall, an increase in oxidative stress is believed to modify/cause several important physiological functions which includes regulation of blood flow and vascular tone, increased platelet and monocyte adhesion to the endothelium, and control of cellular growth influenced by reactive oxygen species. These singularities finally alter blood vessels diameter and remodelling of the atherosclerotic abrasions [48]. These vascular abnormalities that make structural and mechanical changes could offer important insights into the development and progression of vasculature- end-organ damage in many cardiovascular diseases. Therefore, a depressed endothelium dependent vasodilation may be measured an earlier modification, present in essential hypertension and other cardiovascular risk factors, that may lead to the coronary and extra coronary atherosclerotic process by stimulating the proliferation of vascular smooth muscle cells and fibroblasts. Lastly, chronic endothelial dysfunction often may be associated with an erosion or rupture of the atherosclerotic plaque that encourages plaque instability and promotion and chronic vascular syndromes [49].

4. The link with oxidative stress

Oxidative stress has been implicated in the pathophysiology of many cardiovascular conditions, including hypertension. ROS significantly increase the influence of stimulants such as inflammation, radiation, high partial oxygen pressure, advanced age, obesity, and chemical substances. Oxidative stress that increases on a cellular level results in oxidative damage by altering the structure of molecules such as deoxyribonucleic acid, amino acid, protein, lipid, and carbohydrate. A predominantly important radical for cardiovascular biology is superoxide, which is formed by the one-electron reduction of oxygen. Superoxide can serve as both an oxidant and as a reductant and is a progenitor for another ROS. Other radicals are- hydroxyl radical, alkoxyl radicals and lipid peroxyl radical. Other molecules, including hydrogen peroxide, peroxynitrite and hypochlorous acid are not radicals but have strong oxidant properties and are, therefore, included as ROS. Another group of molecules is called the reactive nitrogen species (RNS) which includes nitric oxide, the nitrogen dioxide radical, and the nitro sodium cation. The main sources for oxidative excess in the vasculature are adenine dinucleotide phosphate (NADPH) oxidase (NOX), xanthine oxidase, the mitochondrial and uncoupled NOS [50-52].

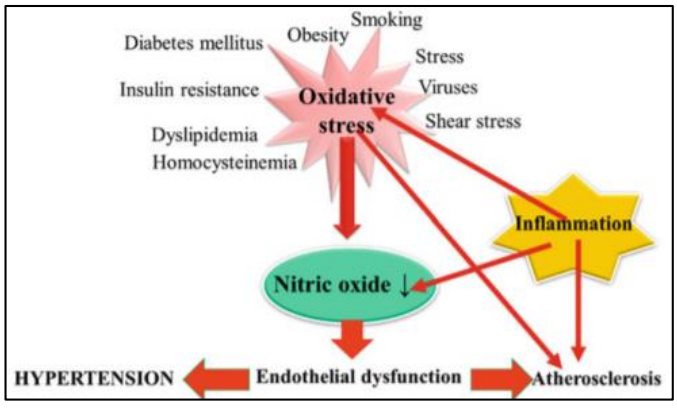

Fig 3: Show hypertension and Atheroscelerosis

5. Pathophysiology of endothelial dysfunction

5.1 Nitric Oxide

Nitric oxide (NO) is one of the most significant vasodilating substances produced and released by the endothelium which functions as a vasodilator, hinders growth and inflammation and has anti-aggregant properties on the platelets. Reduced NO has often been reported in the presence of impaired

endothelial function. It may outcome from reduced action of endothelial NO synthase (eNOS; as an outcome of endogenous or exogenous inhibitors or decrease in the availability of its substrate, L-arginine) and to reduced bioavailability of NO. ROS are known to quench NO with formation of peroxynitrite, which is a cytotoxic oxidant, and through nitration of proteins will affect protein function and therefore endothelial function [53]. Peroxynitrite is an important intermediary of oxidation of LDL, highlighting its proatherogenic role [54]. Moreover, peroxynitrite leads to the deprivation of the eNOS cofactor tetrahydrobiopterin (BH4), leading to "uncoupling" of eNOS [55]. With the help of peroxynitrite decomposition catalyst endothelial dysfunction could be prevented in the diabetic rat [56]. Oxidant excess will also result in the reduction of BH4 with rise in BH2. When this happens, formation of the active dimer of eNOS with oxygenase activity and production of NO is shortened (uncoupling of eNOS). The reductase function of eNOS is activated and more ROS are formed, so NO synthase goes from its oxygenase function producing NO to its reductase function producing ROS, with the subsequent exaggeration of oxidant surplus and deleterious effect on endothelial and vascular function [57]. Oxidative excess is associated to a proinflammatory state of the vessel wall. ROS upregulate both adhesion (VCAM-1 and ICAM-1) and chemotactic molecules (macrophage chemoattractant peptide-1 [MCP-1]). Inflammation reduces NO bioavailability. Undeniably, C-reactive protein (CRP) has been revealed to decrease eNOS activity [58, 59]. The chief source for oxidative excess in the vasculature is NADPH oxidase. Additional sources include xanthine oxidase, the mitochondria and uncoupled NOS [60].

5.2 Asymmetric dimethylarginine

A relatively new and attractive mechanism that leads to reduce NO is asymmetric dimethyl arginine (ADMA), an endogenous competitive inhibitor of eNOS that has been linked to endothelial dysfunction. In human endothelial cells, which were stimulated with the plasma from the patients which had chronic renal disease, inhibition of eNOS was linked with the plasma ADMA levels [61]. ADMA levels were contrarywise connected to endothelium-dependent vasodilation in subjects having hypercholesterolemic condition [62]. It has been concluded that accumulation of this endogenous eNOS inhibitor primes to reduced effective renal plasma flow and increased renovascular resistance and blood pressure [63-64]. ADMA is a product of protein turnover and is removed by excretion through the kidneys or metabolism to citrulline by the enzyme dimethylarginine dimethyl amino hydrolase (DDAH). Recently, overexpression of DDAH was exposed in transgenic mice to increase eNOS activity, decrease ADMA and reduce blood pressure, highlighting the pathophysiologic importance of ADMA [65]. As ADMA is eliminated through renal excretion and degradation by DDAH, it is not surprising that it is increased in patients not only with chronic renal failure but also with other vascular diseases such as in presence of hepatic dysfunction [66-69]. New interest is focusing not only on the elimination but also on the generation of ADMA. Protein-arginine methyltransferases, which produce methylated Arginine's, namely protein-arginine methyltransferase-1, were shown to be up regulated by shear stress, and this upregulation was associated with enhanced ADMA generation [70]. Hypercholesteraemic condition is well proved to be a risk factor for the disease atherosclerosis, accompanying with endothelial dysfunction and there is now also enough proof that elevated ADMA levels are associated with hypercholesterolemia [71]. Plasma ADMA levels were also augmented in elderly hypertensive patients and correlated with age and blood pressure [72]. ADMA levels have been associated with increased cardiovascular risk factors in renal failure, such as CRP, carotid intima-media thickness, concentric left ventricular hypertrophy, and left ventricular dysfunction [73-75]. Moreover, it was found to be a interpreter of acute coronary events overall mortality of the patients with chronic renal failure and mortality of the critical ill patients [76].

5.3 ANG II

Ang II has been indicated in the pathophysiology of chronic renal failure and hypertension. Ang II infusion induces endothelial dysfunction in rats increases ROS by stimulating NAD pH oxidase and triggers vascular inflammation [77-79]. In hypertensive patients, disruption of the renin-angiotensin system (RAS) with angiotensin-converting enzyme (ACE) inhibitors re-establishes healthy endothelial function in contrast to a similar degree of BP lowering with a-blocker, which has no significant effect on endothelium-dependent vasodilation [80, 81].

5.4 Hyperhomocysteinemia

An unconventional cardiovascular risk factor that causes endothelial dysfunction is Hyperhomocysteinemia. Hypercholesterolemia is a pathological condition which is characterized by the presence of very high concentration of cholesterol in the blood. This has been evidenced by different animal models of Hyperhomocysteinemia [82]. Normotensive patients with the condition of Hyperhomocysteinemia display endothelial dysfunction. Folic acid supplements given was able to reduce homocysteine levels and improve endothelial dysfunction in children with chronic renal failure [83]. Various cellular, human and animal studies advocate that homocysteine reduces nitric oxide (NO) bioavailability by oxidative excess or impairment [84]. There is now also evidence that homocysteine may cause ADMA accumulation by inhibition of DDAH [85]. Experimental studies in humans have confirmed that hyperhomocysteinemic condition may lead to the endothelial dysfunction through accumulation of ADMA [86-87]. However, not all studies support this link [88]. These mechanisms may explain the increased cardiovascular risk of patients with Hyperhomocysteinemia. This is of special importance for the patients having chronic renal failure, who often have increased concentration of homocysteine levels, which were shown to predict cardiovascular outcomes in a recent study [89].

6. Therapy of endothelial dysfunction

Endothelial dysfunction may lead to various forms of cardiovascular disease. Treatment of the underlying disease or pathophysical conditions may re-establish endothelial function, even though only in some conditions. In patients with chronic renal failure, renal transplantation restores renal function and may improve endothelial dysfunction [90]. In hypertension, reduction of BP per se does not seem to restore endothelial function. Angiotensin receptor blockers and angiotensin-converting enzyme inhibitors have been shown to be especially beneficial. Mechanisms whereby the blockade of the renin-angiotensin system may improve endothelial function include reduction of oxidative excess and inflammation [91]. In insulin-resistant states and in diabetes,

the mechanisms of endothelial dysfunction are complex and the underlying targets are still not clear. It is interesting that we and others have found that peroxisome proliferator-activated receptor- activators (insulin sensitizers, e.g., the glitazones pioglitazone and rosiglitazone) and peroxisome proliferator-activated receptor-activators exhibit cardiovascular anti-inflammatory and antioxidant properties and correct endothelial dysfunction induced by Ang II (135,136) [92-93]. Another approach for treatment of endothelial dysfunction is to address the components in the disease process that trigger dysfunction of the endothelium. Therefore, decline of homocysteine levels in patients with Hyperhomocysteinemia by supplementation with the folic acid can improve endothelial dysfunction. L-Arginine and tetrahydrobiopterin, as well as tetrahydrobiopterin mimetic may improve endothelial function via increased NO bioavailability [94-95]. However, some studies have not found L-arginine administration to improve endothelial dysfunction [96]. Recently, acetyl salicylic acid has been suggested as an agent that can reduce oxidative stress and improve endothelial function [97]. Statins have proved to have beneficial effects on endothelial dysfunction which may be the result in part of lipid lowering but also of their pleiotropic anti-inflammatory effects [98]. For example, inhibition of LOX-1 by statins and increased eNOS expression and regulation in human coronary artery endothelial cells [99].

7. New research on endothelium

New insights into the regulation of endothelial function will be obtained through greater understanding of the signalling pathways within endothelial cells. Novel research on caveolae and caveolin-1 may be of future interest because endothelial cells exhibit and express high levels of caveolin-1. Caveolin-1 has been implicated in the regulation of eNOS turnover and consequently in NO release as a result of shear stress [100]. A new fascinating aspect of endothelial function is emerging from research on endothelial progenitor cells. These are primitive bone marrow cells that have the ability to mature into endothelial cells and have a physiologic role in repair of endothelial lesions [101]. Levels of circulating endothelial progenitor cells correlate inversely with the degree of endothelial dysfunction in humans at various degrees of cardiovascular risk [102]. It is interesting that eNOS expression by bone marrow stroma cells plays an essential role in the recruitment of endothelial progenitor cells [103]. Therapy with statins increases the number of circulating endothelial progenitor cells [104]. of special interest for patients with chronic renal failure may be the finding that erythropoietin is a potent physiologic stimulus for endothelial progenitor cell mobilization [105]. Moreover, transfer of endothelial progenitor cells may represent a new approach to novel therapy and has been prove to be successful on peripheral ischemia including promoting neovascularization and potentially trans differentiation into cardiomyocytes [101].

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