Uric Acid and Endothelial Dysfunction in Type 2 Diabetes Mellitus: A Biochemical Point of View

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Abstract: Diabetes mellitus (DM) is one of the most common non-communicable diseases globally. Serum uric acid is a strong reducing agent and in humans, over half the antioxidant capacity of blood plasma comes from it. It is widely accepted that uric acid in humans is a major antioxidant that protects cardiac, vascular, and neural cells from oxidative injury, by scavenging hydroxyl radical, singlet oxygen, and peroxynitrite. Hyperuricemia in diabetes leads to endothelial dysfunction and nitric oxide (NO) inhibition. Uric acid reacts directly with nitric oxide in a rapid irreversible reaction resulting in the formation of 6-amino uracil and depletion of NO, which is an endothelial cell derived relaxing factor (EDRF). The probable mechanism by which uric acid may endanger organ damage is through endothelial dysfunction.
Keywords: Uric acid, endothelial dysfunction, Type 2 Diabetes Mellitus

INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases globally [1]. The worldwide prevalence of DM has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 371 million in 2012. Based on current trends, the International Diabetes Federation projects that 438 million individuals will have diabetes by the year 2030 [2]. India is ranked second in the world in diabetes prevalence. In 2011, International Diabetes Federation estimated that India has 61.3 million people living with diabetes [3].

Type 2 DM refers to a group of common metabolic disorders having insulin resistance with relative insulin deficiency or insulin secretory defect [1]. The metabolic dysregulation associated with diabetes mellitus causes secondary pathophysiologic changes in multiple organ systems causing chronic complications. Chronic complications can be divided into vascular and nonvascular complications. The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy, nephropathy, macular edema, sensory, motor and autonomic neuropathy) and macrovascular complications (coronary heart disease, peripheral arterial disease, cerebrovascular disease [1]. Various nonvascular complications also occur. The risk of chronic complications increases as a function of the duration and degree of hyperglycemia [1].

Serum uric acid is a strong reducing agent and in humans, over half the antioxidant capacity of blood plasma comes from it. It is produced by xanthine oxidase (XOR) from xanthine and hypoxanthine, which in turn is produced from purines [4] Humans are unique among mammalian species by having the highest basal blood level of uric acid and the ability to develop hyperuricemia [5]. The known resultant effects of elevated serum uric acid include gout, Lesch Nyhan's syndrome, and uric acid stones [6,7]

It is widely accepted that uric acid in humans is a major antioxidant that protects cardiac, vascular, and neural cells from oxidative injury, by scavenging hydroxyl radical, singlet oxygen, and peroxynitrite [8,9]. The excess breakdown of purines from nucleic acid due to apoptosis and necrosis of vulnerable diabetic end organs lead to uric acid change from antioxidant to oxidant [10]. It loses its antioxidant ability in the hydrophobic complex cellular environment, as in diabetes and can form free radicals

either alone or in combination with peroxynitrite [11,12]. Increased levels of uric acid have been associated with insulin resistance and with established type 2 DM [13,14] Bonakdaran et al found significant correlations between hyperuricemia and serum triglyceride, fasting blood glucose, and glycated haemoglobin [15]. Excretion of uric acid is reduced as insulin can stimulate the urate-anion exchanger and the sodium ion dependent anion co-transporter in brush border membranes of the renal proximal tubule and increase renal urate reabsorption[16-21]. Also ischemia in end organ damage is associated with increased lactate production, which blocks urate secretion in the proximal tubule and increases uric acid synthesis by increasing nucleic acid breakdown and purine (adenine and guanine) metabolism. This increases uric acid and reactive oxygen species (ROS) through the effect of xanthine oxidase [12].

PPARγ is a clinically validated target for treatment of insulin resistance [22]. It is a regulator of adipogenesis, and is involved in expression of anti-inflammatory factor like adiponectin in adipocytes [23]. In a murine model of the metabolic syndrome it was found uric acid entering adipocytes through a uric acid specific transporter (URAT1) could induce downregulation of PPARγ by affecting xanthine dehydrogenase via negative feedback mechanism [24].

SERUM URIC ACID LEVELS AND ENDOTHELIAL DYSFUNCTION

Hyperuricemia in diabetes leads to endothelial dysfunction and nitric oxide (NO) inhibition [25]. Uric acid reacts directly with nitric oxide in a rapid irreversible reaction resulting in the formation of 6-amino uracil and depletion of NO, which is an endothelial cell derived relaxing factor (EDRF). The reduction in endothelial NO level leads to endothelial dysfunction preceding the development of hypertension, arterial stiffness and cardiovascular diseases [26]. Enzyme uricase degrades uric acid to allantoin with the generation of oxidants [27]. Rats administered an uricase inhibitor (oxonic acid) developed mild hyperuricemia, a loss of macula densa nitric oxide synthase, and the development of microvascular disease [27]. Khosla et al showed that hyperuricemic rats have a fall in nitric oxide production that is reversed by allopurinol. They also found there was a linear correlation between serum uric acid and serum NO. Uric acid impaired both basal and vascular endothelial growth factor-induced NO production in cultured endothelial cells [21]. Uric acid also stimulates inflammatory mediators in vascular cells, including C-reactive protein (CRP) and monocyte chemoattractant protein-1 (MCP-1) [28-31]. Sautin et al found soluble uric acid stimulated reactive oxygen species production in cultured mouse mature adipocytes resulting in a decrease in nitric oxide bioavailability, and an increase

in protein nitrosylation and lipid oxidation. This oxidative stress in the adipose tissue is a major cause of insulin resistance and cardiovascular disease [32].

URIC ACID AND VASCULAR COMPLICATIONS

The probable mechanism by which uric acid may endanger organ damage is through endothelial dysfunction [33]. Rats develop high blood pressure after their uric acid level was raised by giving them an inhibitor of uricase. The mechanism of hypertension is by lowering endothelial nitric oxide levels, reducing neuronal nitric oxide synthase in the macula densa of the kidney, and stimulating the renin-angiotensin system [27]. Hyperinsulinemia promotes sodium and water retention, which increases blood volume and pressure. In turn this activates the reabsorption of uric acid resulting in its elevation which in turn increases tubular reabsorption of sodium [12], In type 2 DM, hyperuricemia is associated with early onset or increased progression to overt nephropathy. Hyperuricemia induces inflammatory and remodelling changes within the medullary tubulo-interstitium and glomerulus [12]. Hyperinsulinemia reduces the urinary excretion of uric acid by activating the transporter of uric acid (URAT1), in the proximal tubules of the kidneys [17,20] Exogenous insulin also suppresses urinary uric acid excretion [16,18]. Bonakdaran *et al.* found positive correlation between albumin creatinine ratio and hyperuricemia and confirmed the effect of hyperuricemia on diabetic nephropathy [15]. Serum uric acid in high-normal range is found to be associated with albuminuria and impaired glomerular filtration rate in Chinese type 2 diabetic patients [34]. Patterson et al demonstrated that uric acid accelerates the oxidation of mildly oxidized low density lipoprotein (LDL) [35]. Patients with diabetes having cardiovascular disease (CVD), on suppression of xanthine oxidase, showed vascular endothelial cell-dependent dilatation of blood vessels, suggesting that serum uric acid could be a direct marker of the progression of atherosclerosis [36,37]. Hyperuricemia has been associated with elevated circulating endothelin levels, and one of the major sites of the production of uric acid in the cardiovascular system is the vessel wall endothelium [18,38]. Elevated serum uric acid concentrations predict cardiovascular mortality in type 2 diabetic patients independent of conventional risk factors, diabetes-related variables, medication use, and renal function measures [39]. Hyperuricemia has been found to be a strong predictor of stroke with type 2 diabetes mellitus independent of other cardiovascular risk factors [40]. Serum uric acid levels were higher in type 2 diabetes mellitus patients with sudomotor dysfunction and neuropathy than those without these complications [41,42]. Krizova found that uric acid and glucose concentrations are increased in vitreous and serum of patients with diabetic macular edema and concluded glucose and increased levels of uric acid are involved in

the pathogenesis and progression of diabetic retinopathy [43].

Contrary to the above findings Yuan *et al.* concluded subjects with impaired glucose tolerance had higher serum uric acid levels when compared with subjects having normal glucose tolerance and type 2 DM.[44] However, Fang *et al.* described that the serum uric acid level was higher only in diabetic women but not in diabetic men [45]. Another study conducted in Japanese men showed that uric acid is positively associated with metabolic syndrome but negatively associated with diabetes [46].

Whereas, few studies described inverse correlation between serums uric acid level and type 2 DM [47-49]. On the other hand, some studies reported no association between serum uric acid and DM [50-52]. Considering the conflicting data about uric acid in type 2 diabetes, this review on serum uric acid levels in type 2 DM were already disgned.

REVIEW OF LITERATURE

Insulin resistance and abnormal insulin secretion are central to the development of type 2 DM. Insulin resistance precedes an insulin secretory defect but diabetes develops only when insulin secretion becomes inadequate [53]. It has a strong genetic component. The disease is polygenic and multifactorial, since in addition to genetic susceptibility, environmental factors (such as obesity, nutrition, and physical activity) modulate the phenotype. The genes that predispose to type 2 DM are incompletely identified, but most prominent is a variant of the transcription factor 7–like 2 gene that has been associated with type 2 diabetes in several populations. Genetic polymorphisms associated with type 2 diabetes have also been found in the genes encoding the PPAR-γ, inward rectifying potassium channel, zinc transporter, insulin receptor substrate (IRS), and calpain [54].

Glucose homeostasis reflects a balance between hepatic glucose production and peripheral glucose uptake and utilization. Insulin is the most important regulator of this metabolic equilibrium, but neural input, metabolic signals, and other hormones (e.g., glucagon) result in integrated control of glucose supply and utilization. In the fasting state, low insulin levels increase glucose production by promoting hepatic gluconeogenesis and glycogenosis and reduce glucose uptake in insulin-sensitive tissues (skeletal muscle and fat), thereby promoting mobilization of stored precursors such as amino acids and free fatty acids (lipolysis). Glucagon, secreted by pancreatic alpha cells when blood glucose or insulin levels are low, stimulates glycogenosis and gluconeogenesis by the liver and renal medulla. Postprandial, the glucose load elicits a rise in insulin and fall in glucagon, leading to a reversal of these processes. Insulin, an anabolic hormone, promotes the storage of carbohydrate and fat and protein synthesis. The major portion of postprandial glucose is utilized by skeletal muscle, an effect of insulin-stimulated glucose uptake. Other tissues, most notably the brain, utilize glucose in an insulin-independent fashion [1].

Pathophysiology of dm

Type 2 DM is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and abnormal fat metabolism. In the early stages of the disorder, the pancreatic beta cells compensate by increasing insulin output. As insulin resistance and compensatory hyperinsulinemia progress, gradually a decline in insulin secretion occurs leading to overt diabetes with fasting hyperglycemia. Ultimately, beta cell failure ensues [1]

Mechanism of insulin action

Insulin binds to specific insulin receptors on plasma membrane. The human insulin receptor is a heterotetramer comprising of two alpha and two beta subunits. Alpha subunit is located on the outer surface of plasma membrane and contains the site for insulin binding. The beta subunit extends intracellularly and contains intrinsic tyrosine kinase. Binding of insulin to receptor, causes conformational change in the receptor, resulting in the activation of tyrosine kinase, that catalyses the phosphorylation of tyrosine kinase residues of other intracellular proteins which include IRS-1, IRS-2, IRS-3,IRS-4 and others. Most of these transducer proteins contain one or more Src homology 2 (SH2) domains. SH2 containing proteins include phosphatidylinositol 3-kinase (PI3K) and growth factor receptor- bound protein 2, both of these mediate downstream signal transduction events. Insulin also stimulates mitogen activated protein (MAP) kinase cascade via Ras. In addition, PI3-K activates atypical protein kinase C (aPKC) via akt, which regulates glucose transport by modulating translocation of GLUT4 to the plasma membrane. Akt also phosphorylates and inactivates GSK-3 (glycogen synthase kinase-3) thereby enhancing glycogen synthesis [55].

Insulin resistance and endothelial dysfunction

Insulin resistance, the decreased ability of insulin to act effectively on target tissues especially muscle, liver, and fat where insulin sensitive GLUT 4 are present, is a prominent feature of type 2 DM. Supranormal levels of circulating insulin tries to normalize the plasma glucose. This impairs glucose utilization by these and increases hepatic glucose output; both effects contribute to the hyperglycemia. Increased hepatic glucose output predominantly accounts for increased FPG levels, whereas decreased peripheral glucose usage results in postprandial

hyperglycemia. Glucose metabolism in insulin-independent tissues is not altered in type 2 DM [56].

Insulin receptor levels and tyrosine kinase activity in skeletal muscle are reduced, secondary to hyperinsulinemia. "Postreceptor" defects in insulin-regulated phosphorylation/ dephosphorylation appear to play the predominant role in insulin resistance. Other abnormalities include the accumulation of lipid within skeletal myocytes, which may impair mitochondrial oxidative phosphorylation and reduce insulin-stimulated mitochondrial adenosine triphosphate (ATP) production. Impaired fatty acid oxidation and lipid accumulation within skeletal myocytes also may generate reactive oxygen species such as lipid peroxides. Hyperinsulinemia may increase the insulin action using the MAP kinase pathways, potentially accelerating diabetes-related conditions such as atherosclerosis [1].

The increased adipocyte mass in visceral obesity associated with type 2D M leads to increased levels of circulating free fatty acids and other fat cell products like nonesterified free fatty acids, retinol-binding protein 4, leptin, tumor necrosis factor (TNF), resistin, and adiponectin. In addition to regulating body weight, appetite, and energy expenditure, adipokines also modulate insulin sensitivity [1]. The increased production of free fatty acids and some adipokines may cause insulin resistance in skeletal muscle and liver, as free fatty acids impair glucose utilization in skeletal muscle, promote glucose production by the liver, and impair beta cell function. The production of adiponectin by adipocytes, an insulin-sensitizing peptide, is reduced in obesity, and this may contribute to hepatic insulin resistance. Adipocyte products and adipokines also produce an inflammatory state and markers of inflammation such as IL-6 and CRP are often elevated in type 2 DM. Inhibition of inflammatory signaling pathways such as the nuclear factor kappa B (NF-kB) pathway appears to reduce insulin resistance [56].

In type 2 DM, insulin resistance in the liver reflects the failure of hyperinsulinemia to suppress gluconeogenesis, which results in fasting hyperglycaemia and decreased glycogen storage by the liver in the postprandial state. As a result of insulin resistance in adipose tissue, lipolysis and free fatty acid flux from adipocytes are increased, leading to increased lipid [very low density lipoprotein (VLDL) and triglyceride] synthesis in hepatocytes. This lipid storage or steatosis in the liver may lead to nonalcoholic fatty liver disease. This is responsible for the dyslipidemia found in type 2 DM [elevated triglycerides, reduced high-density lipoprotein (HDL), and increased low-density lipoprotein (LDL) [57].

COMPLICATIONS OF DM
Diabetic ketoacidosis (DKA) and hyperglycaemic hyperosmolar state (HHS) are acute complications of diabetes. DKA was formerly considered a hallmark of type 1 DM, but also occurs in type 2 individuals. HHS is primarily seen in individuals with type 2 DM. Both disorders are associated with absolute or relative insulin deficiency, volume depletion, and acid-base abnormalities. DKA and HHS exist along a continuum of hyperglycaemia, with or without ketosis. Both disorders are associated with potentially serious complications if not promptly diagnosed and treated [1].

VASCULAR COMPLICATIONS OF DM
The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications. The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy and nephropathy) and macrovascular complications [coronary heart disease (CHD), peripheral arterial disease (PAD), cerebrovascular disease]. Nonvascular complications include problems such as gastroparesis, infections, and skin changes. Long-standing diabetes may be associated with hearing loss [1].

Table-1: Chronic Complications of Diabetes Mellitus

MICROVASCULAR
Eye disease
Retinopathy (nonproliferative/proliferative)
Macular edema
Neuropathy
Sensory and motor (mono- and polyneuropathy)
Autonomic
Nephropathy
MACROVASCULAR
Coronary heart disease
Peripheral arterial disease
Cerebrovascular disease
Other
Gastrointestinal (gastroparesis, diarrhea)
Genitourinary (uropathy/sexual dysfunction)
Dermatologic
Infectious
Cataracts
Glaucoma
Periodontal disease
Hearing loss

The risk of chronic complications increases as a function of the duration and degree of hyperglycemia; they usually do not become apparent until the second decade of hyperglycemia. Since type 2 DM often has a long asymptomatic period of hyperglycemia, many

individuals with type 2 DM have complications at the time of diagnosis[1].

Blindness can occur and is primarily the result of progressive diabetic retinopathy and clinically significant macular edema. Diabetic retinopathy is classified into two stages: nonproliferative and proliferative [1].

Diabetic nephropathy is one of the leading causes of ESRD. Both microalbuminuria and microalbuminuria are found in individuals with DM. The mechanisms by which chronic hyperglycaemia leads to ESRD, involve the effects of soluble factors (growth factors, ang II, ET, advanced glycation end products (AGEs)), hemodynamic alterations in the renal microcirculation (glomerular hyper filtration or hyper perfusion, increased glomerular capillary pressure), and structural changes in the glomerulus (increased extracellular matrix, basement membrane thickening, mesangial expansion, fibrosis). Some of these effects may be mediated through angiotensin II receptors. Microalbuminuria is defined as 30–299 mg/d in a 24-h collection. Or 30–299 μg/mg creatinine in a spot collection and microalbuminuria (>300 mg/d or > 300 μg/mg creatinine) [1].

It may manifest as polyneuropathy, mononeuropathy, and/or autonomic neuropathy. Both myelinated and unmyelinated nerve fibers are lost. The most common form of diabetic neuropathy is distal symmetric polyneuropathy, presenting with distal sensory loss [1].

The most prominent gastrointestinal symptoms are delayed gastric emptying (gastroparesis) and altered small- and large-bowel motility (constipation or diarrhea). Hyperglycemia, alongwith parasympathetic dysfunction secondary to chronic hyperglycemia is important in the development of gastroparesis [1].

There is a marked increase in incidence of peripheral arterial disease (PAD), cardiac heart failure, cardiac heart disease (CHD), myocardial infarction, and sudden death in DM. Individuals with type 2 DM have elevated levels of plasminogen activator inhibitors (especially PAI-1) and fibrinogen, which enhances the coagulation process and impairs fibrinolysis, thus favoring the development of thrombosis. Diabetes is also associated with endothelial, vascular smooth-muscle and platelet dysfunction. In addition to CHD, cerebrovascular disease is increased in individuals with DM (threefold increase in stroke)[1].

Foot ulcers and infections are common due to several pathogenic factors like neuropathy, abnormal foot biomechanics, PAD, and poor wound healing. The most common skin manifestations of DM are protracted wound healing and skin ulcerations, leading to diabetic dermatopathy [1]. Individuals with DM have a greater frequency and severity of infection like candida and other fungal infections, due to abnormalities in cell-mediated immunity and phagocyte function associated with hyperglycemia, as well as diminished vascularization [1].

ENDOTHELIAL DYSFUNCTION

The endothelium is the innermost layer of blood vessels, thus it is the largest organ in the body. One of the key functions of the endothelium is to ensure adequate blood flow, which is regulated by the secretion of diverse substances [58]. The endothelium acts to maintain vascular homeostasis through multiple complex interactions with cells in the vessel wall and lumen [59]. The endothelium regulates vascular tone by balancing production of vasodilators, including NO, prostacyclin, endothelium-derived hyperpolarizing factor, C-type natriuretic peptide and vasoconstrictors such as endothelin-1 (ET-1), thromboxane A2, angiotensin II (Ang II), ROS. NO is the major contributor to endothelium-dependent relaxation in conduit arteries, whereas the contribution of endothelium-derived hyperpolarizing factor predominates in smaller resistance vessels [60]. Furthermore, the endothelium controls blood fluidity and coagulation through the production of factors that regulate platelet activity, the clotting cascade, and the fibrinolytic system. Finally, the endothelium has the capacity to produce cytokines and adhesion molecules that regulate and direct the inflammatory process [59].

Physiological impairments that link DM with a marked increase in atherosclerotic vascular disease, include platelet hyper-reactivity, a tendency for negative arterial remodeling, acquires prothrombotic activity, impaired fibrinolysis, increased inflammation, and loss of modulatory role of endothelium known as endothelial dysfunction [61].

Pathophysiology of endothelial dysfunction

Due to abnormal vasoreactivity endothelial cells may adopt a pro-thrombotic phenotype, portending an elevated risk of various complications in diabetic individuals. Furthermore, when exposed to certain pathogenic proinflammatory stimuli, the endothelium expresses leukocyte chemotactic factors, adhesion molecules, and inflammatory cytokines. The term "endothelial dysfunction" refers to this broad alteration in endothelial phenotype that may contribute to the development and clinical expression of atherosclerosis. While the precise mechanisms remain to be elucidated, endothelial dysfunction appears to participate in a "positive feedback loop" in which inflammatory factors promote monocyte and T-cell adhesion, foam cell formation, extracellular matrix digestion, and vascular smooth muscle migration and proliferation leading to

atherosclerotic plaque formation [59]. Dysfunction of the endothelium can be considered when its properties, either in the basal state or after stimulation, have changed in a way that is inappropriate with regard to the preservation of organ function. Basement membrane synthesis may be altered, resulting in changes in cell–matrix interactions [62].

Table-2: Normal Functions of the Vascular Endothelium and a Partial List of Factors Elaborated and Regulated by the Endothelium to Maintain Vascular Homeostasis [6].

Maintenance of vascular tone
Nitric oxide
Prostaglandins (prostacyclin [PGI2], thromboxane A2 [TxA2])
Endothelial hyperpolarizing factor
Endothelin-1
Angiotensin II
C-type natriuretic peptide
Balancing blood fluidity and thrombosis
Nitric oxide
Tissue plasminogen activator
Heparins
Thrombomodulin
Prostaglandins
Plasminogen activator inhibitor-1 (PAI-1)
Tissue factor
Von Willibrand's factor
Control of the vascular inflammatory process
Monocyte chemotactic factor-1 (MCP-1)
Adhesion molecule expression (VCAM-1, ICAM-1, selectins)
Interleukins 1, 6, and 18
Tumor necrosis factor
ICAM-1 _ intercellular adhesion molecule-1; VCAM-1 _ vascular cell adhesion molecule-1.

Type 2 DM is characterised by a decreased glucose transport, which may put an increased demand on both the pancreas and the vascular system, and endothelial dysfunction might thus develop in parallel to hyperglycaemia. Various hypotheses have been put forward to explain the adverse effects of diabetes on the vascular system via an effect of hyperglycaemia on vascular cells, in particular the endothelium.

- High concentrations of glucose accelerate the spontaneous formation of glucose adducts to proteins and other amine-containing molecules. Such early non-enzymatic glycation products are reversible like glycohaemoglobin. These so-called 'Amadori products' become converted to irreversibly modified cross-linked condensation products of glucose and lysine or arginine residues, so-called (AGEs). Irreversible AGE formation is a slow and complex process in which glycation, glycoxidation and auto-oxidative glycosylation occurs. AGEs are encountered in vivo in many different proteins, such as collagen, albumin and plasma lipoproteins. They are also present on red blood cells. AGE formation is greatly accelerated by hyperglycaemia. In addition to glucose, other dicarbonyl sugars, such as methyl-glyoxal, forms spontaneously from triose-phosphates, also modifies proteins and subsequently contribute to the formation of AGEs. Some AGEs are cleared by the kidney and rest accumulate in skin, arteries, kidney and blood of diabetic patients. AGE-adducts can also form on lipids and lipoproteins and increase lipoprotein oxidisability and atherogenicity. Molecules bearing AGEs acquire new properties. AGE-adducts are oxidants; in addition, they interact with and cross-link basement membrane components, thus impairing proper functioning of the basement membrane and stimulating interaction of mononuclear cells with the modified tissue. The appearance of AGEs into the extracellular matrix interferes with endothelial functioning at different levels [62].

- AGEs inhibit a normal network formation by type IV collagen.
- They decrease heparan sulphate proteoglycan binding by vitronectin and laminin..

- They can quench nitric oxide during its passage from the endothelial cell to smooth muscle cells, with loss of its vasodilating and antiproliferative actions.
- In addition to their direct interaction with matrix proteins, AGEs bind to specific cellular receptors, by which they activate endothelial cells, monocytes and mesangial cells [1, 62].

Yan *et al.* suggested that the generation of reactive oxygen species plays a crucial role in endothelial activation by AGEs and causes the activation of NF-kB. The binding of AGEs to their cellular receptor in macrophages, has been shown to induce the synthesis of interleukin-1, tumour necrosis factor-a, and insulin-like growth factor-1, and, in endothelial cells, to increase permeability and to induce tissue factor, vascular cell adhesion molecule-1, heme oxygenase and interleukin-6 expression. AGE-induced TNFα formation activates endothelial cells and thus affects their antithrombotic properties, margination and extravasation of leukocytes and also induces insulin resistance. The cellular activation of endothelial cells by AGEs leads to the possible formation of intracellular AGEs [62].

- Glucose-induced impairment of intracellular metabolic regulation. According to these hypotheses, possible targets of the dysregulation caused by elevated cellular glucose concentrations include: alterations in the cellular redox state by an altered NADH/NAD+ (nicotinamide adenine dinucleotide) ratio and accumulation of sorbitol by increased activity of the sorbitol pathway, catalysed by aldose reductase and sorbitol dehydrogenase respectively. Increased sorbitol concentration alters redox potential, increases cellular osmolality, generates reactive oxygen species, and likely leads to other types of cellular dysfunction [62].
- A hyperactive sorbitol pathway may deplete the cellular niconitamide adenine dinucleotide phosphate (NADPH) pool, which is required for nitric oxide generation and to replenish glutathione. Hyperglycaemia also reduces the activity of the pentose phosphate pathway in endothelial cells, further impairing NADPH generation under conditions of increased demand. As a consequence, oxidants may decrease the cellular glutathione level, which may in turn affect the activities of protein tyrosine kinases,

the activity of which is influenced by the redox state of their sulphydryl groups [1,62].

- A fourth hypothesis proposes that hyperglycemia increases the formation of diacylglycerol through increased glycolysis, leading to activation of protein kinase C (PKC) in particular the PKC-bII isotype. PKC alters the transcription of genes for fibronectin, type IV collagen, contractile proteins, and extracellular matrix proteins in endothelial cells and neurons [1].
- A fifth theory proposes that hyperglycemia increases the flux through the hexosamine pathway, which generates fructose-6-phosphate, a substrate for O-linked glycosylation and proteoglycan production. The hexosamine pathway may alter function by glycosylation of proteins such as endothelial nitric oxide synthase or by changes in gene expression of transforming growth factor (TGF) or PAI-1. As the TGFb1 gene is strongly activated by PKC. TGFb stimulates the accumulation of matrix proteins by enhancing, in mesangial cells and other types of renal cells, the synthesis of various matrix proteins, such as type I and IV collagens, fibronectin and proteoglycans, and by reducing their proteolysis. At the same time, TGFb inhibits the proliferation of various cell types including glomerular endothelial cells. This may reduce the ability of endothelial cells to regenerate after injury. Such structural alterations may contribute to diabetic glomerulosclerosis in kidneys. High glucose is believed to influence endothelial cells indirectly by the synthesis of growth factors in adjacent cells, in particular transforming growth factor-b (TGFb) and vascular endothelial growth factor (VEGF) and by acting on the coagulation cascade, which generates thrombin, a potent cell activator, and fibrin, which may also have stimulatory effects on endothelial function [1,63].
- A possible unifying mechanism is that hyperglycemia leads to increased production of reactive oxygen species or superoxide in the mitochondria; these compounds may activate all of the pathways described above. Reactive oxygen species may modify endothelial function by a variety of mechanisms. These include direct effects on the endothelium such as peroxidation of

membrane lipids, activation of transcription factors like NFkB, leading to the upregulation of adhesion molecules to platelets and leucocytes, and interference with the availability of nitric oxide; and indirect effects such as increasing the oxidation of low-density lipoprotein, the formation of AGEs, and the activation of platelets and monocytes. Hyperglycaemic pseudohypoxia, glucose auto-oxidation and AGE formation may be important determinants of increased oxidative stress. In addition, hyperglycaemia may impair endothelial free radical scavenging by reducing the activity of the pentose phosphate pathway and thus decreasing the availability of NADPH to the glutathione redox cycle [63]. Furthermore, peroxynitrite leads to degradation of the eNOS cofactor tetrahydrobiopterin (BH4), resulting in an uncoupling of eNOS activity. Oxidant excess also results in reduction of BH4 to 7,8 dihydrobiopterin, which leads to decreased formation of the active dimer of eNOS, oxygenase activity and curtailed production of NO. Under these conditions, the reductase function of eNOS is activated to produce more ROS: eNOS shifts from an oxygenase that produces NO to a reductase that produces ROS, with consequent exaggeration of oxidant excess and its deleterious effects on endothelial and vascular function [58].

Hyperglycemia might not be the sole factor responsible for endothelial dysfunction in diabetes mellitus. Other factors such as dyslipidemia, elevated free fatty acids (FFAs), inflammation, and insulin resistance can cause endothelial dysfunction [63].

Endothelial dysfunction and insulin resistance

Insulin's resistance action on the lipid metabolism is associated with the increase in the free fatty acid (FFA) concentrations in plasma, resulting in the induction of oxidative stress and inflammation.[64] The action of FFAs on PI3K–mediated mechanisms may diminish NO release. FFAs may also decrease NO bioavailability through 2 other important mechanisms. First, NO bioavailability may be decreased by oxidative stress, which is increased by obesity and type 2 diabetes mellitus. These conditions not only increase generation of ROS but also lead to oxidative damage to lipids, proteins, amino acids, and DNA [63].

Adipocytes constitutively express the proinflammatory cytokine TNFα, which is increased in insulin resistance. Diabetes itself is inflammatory conditions, as indicated by increased plasma concentrations of CRP, interleukin-6 (IL-6), and PAI-1. Two mechanisms might promote inflammation. First, chronic high glucose increases both oxidative stress and inflammatory changes. Second, increases in TNF α and IL-6 associated with type 2 DM might interfere with insulin action by suppressing insulin signal transduction [63]. The atheromatous lesions contain immune cells (mast cells, T cells, macrophages) that when activated produce inflammatory cytokines. The hemodynamic profile, the retention of LDL in the arterial wall, and the oxidation of LDL may initiate an inflammatory response in the arterial wall. The cytokines present in the atherosclerotic lesions promote a type 1 helper T (Th1) response. The most powerful pro-inflammatory cytokines are CRP and IL-6, and the anti-inflammatory cytokines IL-10 and the TGF-β [64].

Endothelial cells express the cognate insulin receptor (IR), which belongs to a family of membrane-bound receptors with intrinsic tyrosine kinase activity, whose ligands include growth factors such as insulin-like growth factor-1, vascular endothelial growth factor, platelet-derived growth factor, and epidermal growth factor. In addition to crucial metabolic actions, insulin plays a critical role in the maintenance of physiological endothelial function through its ability to stimulate NO release via a cascade of signaling that involves activation of the PI3K-Akt axis and the downstream serine phosphorylation of endothelial NO synthase (eNOS). In addition to NO-dependent vasodilatory actions, insulin stimulates endothelial release of the vasoconstrictor ET-1. Thus, insulin has multiple opposing hemodynamic actions. Insulin resistance is characterized by specific impairment in PI3K-dependent signaling pathways, whereas other insulin-signaling branches, including Ras/MAP kinase dependent pathways, are unaffected. In addition, metabolic insulin resistance is usually paralleled by a compensatory hyperinsulinemia to maintain euglycemia, which overdrives unaffected mitogen-activated protein kinase dependent pathways in endothelium. This may lead to decreased production of NO and increased secretion of ET-1, a characteristic of endothelial dysfunction [64]. Insulin and insulin precursors may increase plasma levels of PAI-1. High PAI-1 levels inhibit fibrinolysis and facilitate the persistence of fibrin, which may damage the endothelium. Insulin can stimulate vascular smooth muscle cell proliferation, increase vascular permeability to macromolecules. Insulin may indirectly increase the risk of atherosclerotic disease through its associations with hypertension and dyslipidemia (high triglyceride and low HDL-cholesterol levels) [63].

Diabetes in humans is associated with activation of the coagulation cascade at multiple levels. Normally the release of NO and prostaglandlin PGI2 increases the activity of guanylate- and adenylate-

cyclase, respectively, raising cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate levels. This is followed by inhibition of platelet aggregation and thrombosis. The endothelial expression and presentation of the cell surface protein thrombomodulin leads to inhibition of thrombosis. Thrombomodulin binds thrombin, causing a configurational change that inhibits the conversion of fibrinogen into fibrin and permits the activation of protein C by thrombin, followed by inactivation of factor Va and VIIIa. Moreover, the endothelium enables fibrinolysis in order to ensure vascular patency and perfusion. After secretion of tissue plasminogen activator (tPA) by the endothelium, active plasmin is formed and this leads to fibrin degradation. In contrast, the endothelium and other tissues secrete PAI-1, which inhibits tPA and functions as an anti-fibrinolytic agent [58]. All this is reversed with endothelial dysfunction. The secretion of tissue factor pathway inhibitor, an endothelium-derived protein, may be increased. The activation of the coagulation cascade is accentuated in patients with relatively severe endothelial dysfunction. Hyperglycaemia is thought to activate the coagulation cascade by various mechanisms, such as non-enzymatic glycation which may decrease antithrombin III (AT III) activity, AGE formation, which may increase tissue factor activity, impairment of heparan sulphate synthesis which decreases AT III co-factor activity, and increased oxidative stress. Thrombin and fibrin fragments are strong stimulators of endothelial cells. Therefore, repeated activation of coagulation may cause overstimulation of endothelial cells and endothelial dysfunction[58].

Type 2 DM is associated with slight increases in the levels of acute-phase proteins, such as CRP and fibrinogen, which in turn are markers of an adverse cardiovascular prognosis under various circumstances [62].

In type 2 DM, increased calpain (calcium-dependent protease) activity in response to hyperglycemia may play a role in diabetic vascular disease. Immunoprecipitation studies revealed that glucose induces loss of NO via a calpain-dependent decrease in the association of hsp90 with endothelial NOS. Inhibition of calpain activity decreases endothelial cell surface expression of the pro-inflammatory adhesion molecules ICAM-1 and VCAM-1 during hyperglycemia and inhibition of PKC activity reduces leukocyte-endothelium interactions by suppressing surface expression of endothelial cell adhesion molecules in response to increased oxidative stress [65].

It is tempting to speculate that loss of endothelial-dependent vasodilation and increased vasoconstrictors might be etiological factors of hypertension. Moreover loss of activity and/or quantity of endothelium-bound protein lipase activity may contribute to hyperlipidemia, which is typical of the insulin resistance syndrome. A synergistic interaction and vicious cycle may exist in which endothelial dysfunction contributes to insulin resistance and vice versa. Endothelial NOS infiltrates into caveolae, which are cholesterol-rich invaginations present in endothelial cells and VSMC that decrease vasoconstrictive responses to ang II, endothelin and constitutive endothelial NOS activity. Addition of oxidized LDL to cultured endothelial cells disrupts the caveolae complex and is thought to be associated with decreased endothelial NOS activity and endothelial dysfunction. HDL cholesterol can prevent the oxidized LDL–mediated decrease in cholesterol in caveolae, prevent the translocation of endothelial NOS and caveol in from caveolae, and prevent the decrease in responsiveness to acetylcholine. These effects occur because HDL cholesterol donates cholesterol to the caveolae complex. These cellular events are consistent with the proatherogenic effects of LDL cholesterol and oxidized LDL cholesterol and the protective effects of HDL cholesterol [65].

Clinical utility of studying endothelial function

Endothelial vasomotor function can readily be measured in the coronary and peripheral circulations and that systemic markers of endothelial phenotype can be measured in blood. Study of endothelial function may prove advantageous because they provide insight into vascular function. The assessment of endothelium dependent vasodilation has emerged as an accessible indicator of endothelial health. In particular, stimuli that increase production of endothelium-derived NO have proven useful in assessing endothelium-dependent vasodilation in humans. Measurement of serum markers of inflammation (e.g., CRP) is another promising approach to this issue, but may not reflect the susceptibility of the vasculature to the adverse effects of systemic inflammation. It is possible that the state of the endothelium may reflect the degree to which the vasculature has been altered by inflammatory stimuli, and, thus, may provide additional prognostic information. Also unknown is the potential role of more specific serum markers of endothelial dysfunction such as PAI-1, ET, and adhesion molecules (ICAM-1, VCAM-1). Thus, evaluation of endothelial function could be advantageous in prevention of both primary and secondary events. A paradigm shift from the current reactive, symptom-based, screening system looking for active disease to a non-invasive, relatively inexpensive, screening system based on vascular function would also be of benefit to both the general health of the population and the already overburdened and costly medical system. Reproducible evaluation of endothelial function is limited to facilities with extensive experience in these techniques [60].

Evaluation of endothelial dysfunction
Nitric oxide and endothelial dysfunction

Nearly all stimuli that produce vasodilatation do it through NO, a volatile gas, biologically active, present practically in all tissue and due to its low molecular weight and its lipophilic properties it diffuses easily across cell membranes. The NO crosses the endothelial intima and reaches the smooth muscular tissue of the arterial wall, and, through nitrosilation from the guanylate-cyclase degrades the guanosine triphosphate (GTP) releasing cGMP, which in turn regulates the cytosolic Ca2+ and causes smooth muscle fiber relaxation and therefore vasodilatation [67]. (14) NO is not only involved in the control of vasomotor tone but also in vascular homeostasis and neuronal and immunological functions. Endogenous NO is produced through the conversion of the amino acid, l-arginine to l-citrulline by the enzyme, NOS [65], requiring O2 and NADP coenzyme, essential in redox process. BH4 accelerates this process, which is favored by other cofactors like flavin-adenine dinucleotide, and thiol groups like cysteine and reduced glutathion. Three NOS isoenzymes are known, two constitutive and low-production, NOS-I from neurological tissue and NOS-III from endothelial cells, both respond to agonist that increase intracellular Ca2+. The other one, inducible NOS-II, is specially expressed in macrophage and endothelial cells due to the effect of pro-inflammatory cytokines and can release several times more NO than the constitutives NOS. Both constitutives and inducible NOS are in the endothelial cells. Constitutives NOS produces NO for short periods when it is induced by vasodilators like acetylcholine or bradykinin. Inducible NOS synthesize NO for longer periods in a constant manner when the stimulus comes from pro-inflammatory cytokines like TNF-α) [66].

In vascular endothelial cells, insulin stimulates NO production in a dose-dependent fashion through the PI-3k/protein kinase B (Akt) pathway. In arteriolar muscle cells, NOS activation by insulin is dependent on a normally responsive IRS-1/PI-3 pathway. Conversely, insulin resistance at this level impairs insulin-mediated vasodilation and results in endothelial dysfunction. Because muscle is a key site of insulin resistance in diabetes, it can be suggested that the endothelial cells involved are mainly those of skeletal muscle. NO metabolite concentrations are reduced by hyperinsulinemia despite the increase of NO metabolite synthesis, as insulin exerts not only stimulatory effect on NO metabolite production but also increases NO metabolite removal. High glucose also inhibits NOS activity, through a PKC associated mechanism [67].

The diabetic subjects were treated with hypoglycaemic agents and some of them, were also treated with pressure-lowering agents and with statins. These drugs may affect NOS activity, which shows a decreased NO metabolite production, suggesting that, despite therapy, they had an impaired NOS activity. The increase of inducible NOS by endotoxin and/or inflammatory states is associated with impaired insulin stimulated muscle glucose uptake [68]. NO apart of being a vasodilator, also reduces vascular permeability and the monocyte and lymphocyte adhesion molecules synthesis. NO also reduces platelet aggregation, tissue oxidation, tissue inflammation, activation of thrombogenic factors, cell growth, proliferation and migration, also it inhibits proatherogenic and pro-inflammatory cytokines expression and it favors fibrinolysis. NFkB inhibitor (I-kB) is also expressed by NO. All these factors reduce atherogenesis and its complication. For this reason NO is considered the antiatherogenic molecule [67].

The endothelial cell releases ang-II (AII) as an antagonist of NO by means of hydrolyzing angiotensin-I by angiotensine converting enzyme (ACE). Through AT1 receptor, ang II causes vasoconstriction and prothrombogenic, oxidizing and antifybrinolitic effects, and also favors adhesion molecule expression and leukocyte adhesion. Also it stimulates growth and proliferation factors, activates inflammation and incites expression of proinflammatory and proatherogenic cytokines. All these effects allow atherosclerosis to start develop, progress and complicate [66].

Vascular injury in diabetes consequential from hyperglycemia has been associated with oxidative stress that leads to depletion of intracellular glutathione with an augmented plasma extracellular superoxide dismutase which intervenes lipid peroxidation and diabetic complications [68]. Glutathione is the most abundant antioxidant in plasma, reacts directly with NO in a rapid irreversible reaction resulting in the formation of 6-aminouracil and depletion of NO, which is an EDRF. The reduction in endothelial NO level leads to endothelial dysfunction [69]. Elevated concentration of superoxide dismutase causes impairment of eNOS by triggering advanced glycation end products and poly adenosine di nucleotide phosphate (ADP ribose) polymerase [69].

NO is usually generated by NOS, although XOR may also be a contributory source of NO under hypoxic conditions. Since endothelium-derived NO controls vascular tone, prevents adhesion of leukocytes, inhibits aggregation and adhesion of platelets, and reduces intima proliferation, the reduction of NO bioavailability induces endothelial dysfunction and oxidative stress, a key mechanism of vascular risk and dysfunction [70].

High sensitive crp and endothelial dysfunction

CRP is an acute phase protein synthetized by liver in inflammation, which is recognized as a reliable

marker reflecting the inflammatory state 19. There is a clear relationship between inflammation and thrombosis, each influencing the other. Inflammatory cytokines induce procoagulant molecules in endothelial cells. Interleukin-6 increases plasma concentrations of CRP, fibrinogen and PAI-1 in the liver. CRP amplify immunological response inducing leukocyte adhesion molecules and chemokines production in the endothelial cells, and show synergetic action with bacterial polysaccharides inducing monocyte grow factors [71].

C-reactive protein CRP promotes endothelial cell inflammation and atherosclerotic development by stimulating the expression of PAI-1, VCAM, ICAM and E-selectin, increases the release of MCP-1 and IL-8, and suppresses prostacyclin production in endothelial cells. CRP also attenuates endothelial progenitor cell survival and differentiation. Nystrom and co-workers indicated that persistent endothelial dysfunction is related to elevated CRP levels in type 2 diabetic patients. A recent study demonstrated that one of the serum leptin- interacting proteins is CRP, the expression of which can be stimulated by leptin in human hepatocytes [71].

In normal individuals, insulin has been shown to suppress several pro-inflammatory transcription factors, such as the NFkB and CRP is an important pro-inflammatory marker, has recently been introduced as a new factor of the metabolic syndrome [64].

CRP acts on vascular smooth muscle cells by upregulating the angiotensin type I receptor and stimulating the migration and proliferation of smooth muscle cells, in addition to the production of reactive oxygen species.Inhibition of rennin–angiotensin system (RAS) by angiotensin receptor blocker (ARB) or ACE inhibitor resulted in a reduction of VSMC proliferation. In addition, ang II, via the type-1 (AT1) receptor, stimulates NADPH oxidase and enhances production of ROS, which in turn contributes to endothelial dysfunction by inactivating NO [71]. Secondly, CRP has a direct effect on the endothelial cells and induces the secretion of specific chemokines, particularly MCP, adhesion molecules, and E-selectin, whereas it decreases the expression of NOS, which accelerate atherosclerosis [72].

Although few epidemiological studies have demonstrated that CRP is related to insulin resistance. CRP attenuates insulin signalling through the regulation of (Syk) tyrosine kinase and RhoA in the phosphorylation of the insulin IRS-1, akt, and eNOS in vascular endothelial cells, leading to an imbalance between NO and ET-1 production. Treatment with recombinant CRP causes an increase in phosphorylation at the Ser307 site of IRS-1 in a dose- and time-dependent manner. Moreover, pretreatment with

recombinant CRP restrained the insulin-induced phosphorylation of both Akt and eNOS proteins, decreased intracellular phosphatidyl inositol-3,4,5-triphosphate (PIP3), and suppressed insulin-induced NO_2 production. The RhoA-Rock- JNK (Janus kinase) pathway mediates the inhibitory effect of recombinant CRP on IRS-1 activity. Recombinant CRP upregulated Rho GTPase relative activation in a time-dependent manner. Recombinant CRP stimulates the phosphorylation of JNK, whereas transfection of the dominant negative RhoA cDNA blocked recombinant CRP-stimulated JNK phosphorylation and blocks recombinant CRP-induce IRS-1 phosphorylation at the Ser307. Syk tyrosine kinase has a crucial role in multiple leukocyte intracellular signaling pathways. Syk is also expressed in vascular endothelial cells. CRP causes the tyrosine phosphorylation of Syk. Inhibition of Syk tyrosine kinase Improve the CRP-induced imbalance between NO and ET-1 Production which is associated with endothelial dysfunction [71].

A recent study showed that pure human CRP increases rather than decreases, the bioavailability of NO in blood vessels in vitro [74].

URIC ACID
Uric acid is the end-product of purine nucleotide metabolism in humans. In contrast to many lower vertebrates, human lack uric acid oxidase (uricase), an enzyme which further catalyses uric acid to allantoin, more soluble end product. Humans have higher serum uric acid levels when compared to other mammals due to the lack of uricase. Uric acid is primarily excreted via the urine. The balance between dietary intake, endogenous metabolism of purines and the urinary excretion rate of uric acid determines plasma uric acid levels [73]. Gout, primary enzyme deficiencies, exercise, or diuretic medications and salicylate usage may cause uric acid elevations [74]. Almost all serum uric acid is present in the ionized form, monosodium urate, and only about 5% of urate is protein bound at physiological pH. The definition of hyperuricemia is currently arbitrary and varies from >6 mg/dL (360μmol/l) in women and >7 mg/dL (416μmol/l) in men, to ≥6.5 mg/dL (387μmol/l), or to >8.3 mg/dL (494 μmol/l), regardless of gender. Uric acid levels physiologically and gradually rise during the human lifetime; in female individuals, uric acid levels additionally rise after menopause [73].

Kidneys eliminate two-thirds, and the gastrointestinal tract eliminates one-third of the uric acid load. Urate is filtered completely at the renal glomerulus. However, the normal fractional excretion of uric acid is only 8% to 12%. Therefore, post glomerular reabsorption and secretion are the ultimate factors regulating the amount of uric acid excretion. The proximal tubule is the site of uric acid reabsorption

and secretion. Almost complete reabsorption of urate occurs at the S1 segment of the proximal tubule. However, in the S2 segment of the proximal tubule urate is secreted at a range greater than reabsorption via transporters URAT1, OAT1-4 and -10, the multidrug resistance proteins ABCC4 and ABCG2, and the GLUT 9a and b, and others. Finally, post-secretory resorption occurs at a more distal site of the proximal tubule. Approximately 10% of the filtered urate appears in the urine [73].

Uric acid is a weak acid with 2 dissociation constants. Two factors contribute to its solubility: uric acid concentration and solution pH. However, the solubility of uric acid in urine is primarily determined by urinary pH. The first pKa of uric acid is at a pH of 5.5, resulting in the loss of 1 proton from uric acid and the formation of anionic urate. The second pKa is 10.3, which has no physiological significance in humans. The supersaturation of urine with uric acid occurs when urinary pH is less than 5.5. In contrast, at a pH of more than 6.5 the majority of uric acid is in the form of anionic urate. The solubility of urate salts is affected by the relative concentrations of cations present in the urine. Increased urinary sodium concentrations promote formation of the monosodium urate complex, which is more soluble than undissociated uric acid. Urine is frequently supersaturated with sodium urate but stones of this type are infrequent. However, supersaturation with sodium urate may contribute to calcium oxalate stone formation via heterogeneous nucleation [73].

Uric acid may be derived by endogenous or exogenous routes. The endogenous production of uric acid from purine synthesis, and tissue catabolism under normal circumstances, is relatively constant at 300 to 400mg per day. However, the exogenous pool varies significantly with diet. A diet rich in animal protein contributes significantly to the purine pool and subsequent uric acid formation by a series of enzymatic reaction involving xanthine oxidase as the final step [73].

Renal excretion is the primary mode of uric acid clearance, accounting for two-thirds of its elimination. Intestine, skin, hair and nails account for the remaining one-third of uric acid excretion. In the intestine bacteria catabolise uric acid into carbon dioxide and ammonia, which are then eliminated as intestinal air or absorbed and excreted in the urine [73]. A major site of uric acid production is the microvascular endothelium, the place where LDL oxidation is thought to take place [75].

Uric acid and type 2 diabetes mellitus
The role of xanthine oxidoreductase in endothelial dysfunction

XOR is a molybdoflavoprotein enzyme that exists in two interconvertible functionally distinct forms, namely xanthine dehydrogenase (XD) and xanthine oxidase (XO). XO is generated from XD by proteolysis and catalyzes the two terminal steps of purine degradation (hypoxanthine → Xanthine → Uric acid) in humans. By oxidation of hypoxanthine to xanthine, and xanthine to urate, XO readily donates electrons to molecular oxygen; thereby producing superoxide radical (O_2^-) and hydrogen peroxide. Both XD and XO contain an internal electron transport system that is capable of producing ROS. The physiological substrates, xanthine and hypoxanthine, bind the oxidized enzyme and donate two electrons to the molybdenum cofactor, reducing it from MoVI to MoIV. Substrates are hydroxylated by H_2O at the molybdenum site as the electrons travel via two iron-sulfide residues to flavine-adenine oligonucleotide (FAD). Reduced FAD can be divalently reoxidized by oxygen to produce hydrogen peroxide (H_2O_2), or univalently reoxidized in two steps to generate two equivalents of superoxide radical. Superoxide has been identified as the most probable ROS contributing to endothelial dysfunction. The superoxide radical produced by either XO or XD can bind NO to form peroxynitrite ($OONO^-$), a potent non-radical oxidant species. In particular, XO has been implicated as a source of ROS in the vascular system [70].

Uric acid as a potentially useful antioxidant

Uric acid, together with ascorbic acid, is considered a major water soluble intracellular free radical scavenger, as well as an antioxidant in human plasma, since it can react with superoxide anion, hydrogen peroxide, hydroxyl radical, and particularly peroxynitrite. Uric acid reacts preferentially with peroxynitrite anion, which is a short-lived oxidant species formed by the reaction between NO and superoxide radical, occurring particularly in the vascular endothelium. Peroxynitrite is capable of inducing cell death or abnormal functioning, thus contributing to various forms of endothelial dysfunction. In cell culture systems peroxynitrite induced BH4 oxidation and a decrease in NO generation. In peroxynitrite-treated human plasma, Skinner *et al.* have detected a nitrated uric acid derivative. This product may be involved in human pathophysiology by releasing NO, which could decrease vascular tone and increase tissue blood flow, thereby suggesting a novel beneficial role for uric acid. The relationship between uric acid and NO has also been characterized by another reactive product nitrosated uric accid, identified by mass spectrometry under aerobic conditions, which results from the reaction with NO donors, but not with peroxynitrite. Thus uric acid may act as a vehicle of NO, suggesting that its elevated levels may represent a favourable response to oxidative stress in endothelial dysfunction [70].

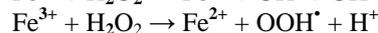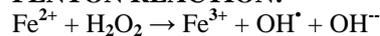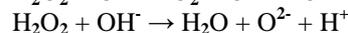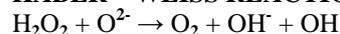**Deleterious effects of uric acid and mechanisms involved**

In overt contrast with the above data, since 2005 experimental and clinical studies have suggested that elevated uric acid levels are associated with a reduction in NO levels with ensuing endothelial dysfunction. Uric acid reduces NO levels in endothelial cell cultures, blocks acetylcholine-induced vasodilatation of aortic rings, and reduces circulating nitrites in experimental animals. Further, rat studies have shown that hyperuricemia-induced hypertension and vascular disease are partially reversed by the supplementation of the NOS substrate, L-arginine. Gersch *et al*. have demonstrated that uric acid reacts directly with NO in a rapid irreversible reaction resulting in the formation of 6- aminouracil, with consequent depletion of NO. This reaction occurs preferentially with NO, even in the presence of peroxynitrite and hydrogen peroxide, and is partially blocked by glutathione, showing a potential mechanism by which uric acid could deplete NO under conditions of oxidative stress, when intracellular glutathione is depleted [26].

There is increased vascular cell apoptosis and inflammatory necrosis due to increased oxidative stress through ischemia-reperfusion in diabetes resulting in with increased purine metabolism by xanthine oxidase. Thus, these leads to increase in serum uric acid levels due to increased oxidative-redox stress and antioxidant "Prooxidant Paradox of Urate Redox Shuttle" where an antioxidant uric acid gets converted to a pro-oxidant. Due to hyperinsulinemia, leptin may induce hyperuricemia, and also insulin increases sodium reabsorption and is tightly linked to urate reabsorption. Additionally, low density lipoproteins such as LDL-cholesterol are capable of being modified and retained within the intima through a process of oxidative modification through free radicals, hypochlorous acid, peroxynitrite, and selected oxidative enzymes such as xanthine oxidase, myeloperoxidase and lipoxygenase [73]. Increased visceral fat accumulations provide excessive free fatty acid in the portal vein, which accelerates the overproduction of very low-density lipoprotein and this causes hypertriglyceridemia. This also accelerates the de novo purine synthesis by NADPH produced in the pentose phosphate pathway which increases the uric acid production [74].

This urate redox shuttle is influenced by on its surrounding environment such as timing (early or late in the disease process), location of the tissue and substrate, acidity (acidic – basic or neutral ph), the surrounding oxidant milieu, depletion of other local antioxidants, the supply and duration of oxidant substrate and its oxidant enzyme. In the accelerated atherosclerotic vulnerable plaque the intima has been shown to be acidic, depleted of local antioxidants with an underlying increase in oxidant stress and ROS and associated with uncoupling of the eNOS enzyme and a decrease in the locally produced naturally occurring antioxidant: eNO and endothelial dysfunction.

Reactions such as the Fenton and Haber- Weiss reactions lead to an elevated tension of oxidative – redox stress.

FENTON REACTION:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^-$$
$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + OOH^{\bullet} + H^+$$

HABER – WEISS REACTION:

$$H_2O_2 + O^{2-} \rightarrow O_2 + OH^- + OH$$
$$H_2O_2 + OH^- \rightarrow H_2O + O^{2-} + H^+$$

The hydroxyl radicals can then proceed to undergo further reactions with the production of ROS through addition reactions, hydrogen abstraction, electron transfer, and radical interactions. Additionally, copper (Cu^{3+} - Cu^{2+} -Cu^{1+}) metal ions can undergo similar reactions with formation of lipid peroxides and ROS. This makes the leakage of iron and copper from ruptured vasa vasorum very important in accelerating oxidative damage to the vulnerable accelerated atherosclerotic plaques, as well as, providing a milieu to induce the serum uric acid antioxidant – prooxidant switch [10].

Uric acid as a risk factor for vascular disease although many experimental and epidemiological data have suggested a possible role for hyperuricemia in inducing endothelial dysfunction, and particularly impaired NO bioavailability, not all studies have shown a causal role for uric acid in the development of endothelial dysfunction [70].

Studies related to significance of no, uric acid, crp in type 2 diabetes mellitus

The endothelial dysfunction that has been attributed to oxidative stress, dyslipidemia, accumulation of endogenous inhibitors of NO synthase, genetic factors, and other causes, recent experimental data implicates role of uric acid in this endothelial dysfunction, but data from few studies are controversial.
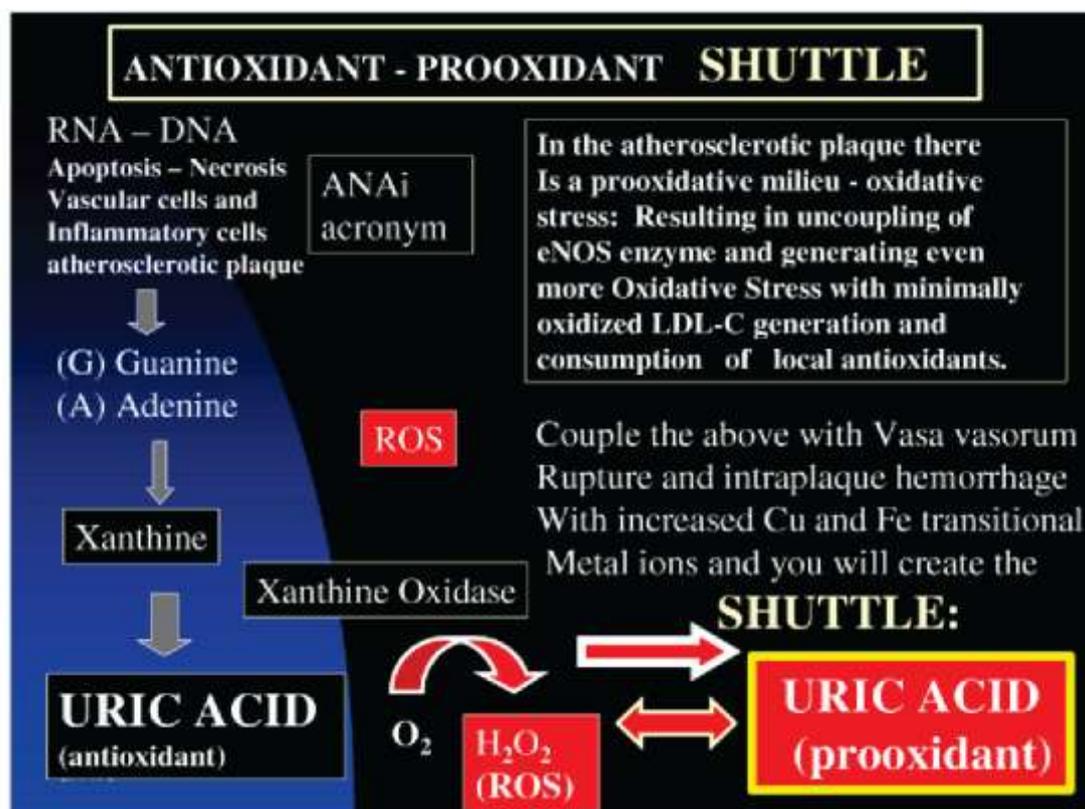
Fig-1: Figure demonstating URATE SHUTTLE [68]

In patients with type 2 diabetes and with hypercholesterolemia but not in patients with essential hypertension, allopurinol, a xanthine oxidase inhibitor lowers uric acid and interacts with anion superoxide generation, improves endothelial dysfunction. In the study of Mercuro *et al.* [76], the beneficial effect of allopurinol could have been a direct consequence of the reduced UA levels rather than of superoxide anions mediated by xanthine oxidase inhibition because of the close correlation found between the amount of that decrease and the improvement of endothelial function. Recent findings suggest that fructose-induced hyperuricemia reduce endothelial NO levels and induce insulin resistance [76].

Khosla *et al.* showed direct linear correlation between serum uric acid and serum NO and found that uric acid impaired both basal and VEGF-induced NO production in cultured endothelial cells, suggesting that hyperuricemia may cause endothelial dysfunction [21]. Waring *et al.* [77] reported that the infusion of uric acid into humans does not impair endothelial function over a 1-hour period. However, these studies did not measure effects of sustained hyperuricemia on endothelial-dependent vasodilatation [77]. Allopurinol treatment for 3 months has been shown to improve FMD in hyperuricemic patients but not in normal subjects [78].

Zoccali *et al.* [79]. Showed all the subjects in their study had no history of a gout attack for at least the previous 4 weeks and lacked any clinical evidence of infection; however the hyperuricemic patients had higher levels of CRP than the controls. Uric acid-induced expression of CRP has been observed previously in human VSMCs and endothelial cells. More importantly, uric acid was found to be associated with several inflammatory markers, including CRP and IL-6, in another population-based study. Thus, these results imply that uric acid may induce endothelial dysfunction and vascular inflammation reaction, which play pivotal roles in the pathogenesis of atherosclerosis. Several data demonstrate that uric acid can stimulate the synthesis of CRP, and that might be one of the mechanisms underlying the endothelial dysfunction. They also found that CRP is a stronger correlate of endothelial function than creatinine, and the observation that the uric acid endothelial function link remains strong in a statistical model that included CRP also suggests that inflammation-independent pathways play a significant role in the putative effect of uric acid on endothelial function [79].

Uric acid could worsen insulin resistance by disturbing insulin-stimulated glucose uptake. Two recent meta-analysis revealed that elevated serum uric acid has been an independent risk factor for the development of type 2 diabetes. Additionally, serum

uric acid could also lead to endothelial dysfunction by inhibiting the bioavailability of NO, the progression of which might cause vascular lesions and even death. Thus, whether serum uric acid is also independently associated with the development of vascular complications and mortality in type 2 DM is essential for its secondary and tertiary prevention [80].

Recently, Kim *et al.* [81]. suggested that elevated serum uric acid was associated with diabetic nephropathy. Zoppini *et al.* [39] found that elevated serum uric acid concentrations independently predicted cardiovascular mortality in type 2 DM. However, Ong *et al.* [82] revealed that serum uric acid did not predict cardiovascular or all-cause mortality in type 2 DM. Various types of study populations and study designs might have contribution to these disparate findings, as shown in table 3 [80]. Thus, the aim of our study was to ascertain the role of serum uric acid in type 2 DM.

Serum uric acid seems to be a graded marker of risk for the development of CHD or CVA and stroke compared with patients with normal uric acid levels and especially those in the lower 1/3 of its normal physiological range.[10]

Study by Strasak *et al.* investigated the association between increased uric acid concentrations in serum and mortality from cardiovascular causes in more than 80,000 Austrian men followed for a median of 13.6 years, and found increased uric acid was associated with increased risk of death from CHD, congestive heart failure (CHF), and stroke [89].

Ito *et al.* [86] showed the incidence of coronary heart disease was significantly higher in the patients with hyperuricemia than in those without during the follow-up observation period. They suggested that high uric acid levels clearly predicted the incidence of diabetic macroangiopathies in the patients with type 2 DM. Rathmann *et al.* [90] reported that high uric acid levels was associated with coronary heart disease in 4,047 patients with type 2 diabetes mellitus according to a cross-sectional study.

Table-3: Characteristics of 9 articles focusing on relationship of SUA and macrovascular disease, microvascular disease, mortality in type 2DM [80].

Lead author's name	Publication year	Study design	duration (yrs)	No of subjects	Study Population	Mean age	men (%)	Complications
Tapp[83]	2003	Case control	/	11247	Australian	≥25	51.4	PVD NA Neuropathy
Tseng[84]	2004	Case control	/	508	Chinese	63.8+10.6	41.3	PVD
Cai [85]	2006	Case control	/	526	Chinese	55.9	60.2	Retinopathy
Zoppini [39]	2009	Cohort	4.7	2726	Italian	67.3+9.6	55.3	CHD-M All cause M
Ong [82]	2010	Cohort	/	1,268	Australian	64.1+10.5	48.6	CHD-& All cause Mortality
Kim [81]	2011	Case control	/	504	Korean	57.3±13.9	47.4	Nephropathy
Ito [86]	2011	Case control	/	1,213	Japanese	64.0+12.0	59.0	CHD PVD CVD Retinopathy Nephropathy Neuropathy
Zoppini [87]	2012	Case control	5.0	1449	Italian	66.1 + 9.9	61.3	Nephropathy
Panero[88]	2012	Cohort	NA	1540	Italian	68.9	NA	All cause CHD Mort Non CHD Mortality

Several other investigators have described elevated serum uric acid to be a risk factor for atherosclerotic disease in patients with diabetes mellitus. Ito *et al.* [86] showed in their study, the eGFR (estimated glomerular filteration rate) significantly decreased in the patients with hyperuricemia compared with those without, and reported the changes in the eGFR between diabetic patients without and with

hyperuricemia, to be significantly different in the glomerular function between the two groups within one year after observation was started.

Tseng *et al.* [84] and Fukui *et al.* [91] reported that the serum uric acid level was elevated, along with increased urinary albumin excretion in the smaller study population with type 2 diabetes using a cross-sectional design. Li *et al.* [48] revealed that the level of serum uric acid was negatively correlated with the eGFR in 1,026 Chinese patients with type 2 DM.

Papanas *et al.* [41] described in patients with type 2 DM, high serum uric acid levels have been linked with macrovascular disease, namely, stroke and PAD while the type 2 DM patients with sudomotor dysfunction exhibit significantly higher serum uric acid than those without sudomotor dysfunction. Serum uric acid levels are elevated in association with the severity of sudomotor dysfunction and they are associated with increased CRP and dyslipidemia. Based on these findings, serum uric acid may be considered a potentially useful additional marker for sudomotor dysfunction [41].

Lehto *et al.* showed independent role of hyperuricemia as a predictor of fatal and nonfatal stroke events in patients with type 2 DM. The risk of stroke was increased twofold among type 2 DM patients with high uric acid (295 mmol/L) compared with those with low uric acid [40].

Choi *et al.* indicated that individuals with pre-diabetes may be at a higher risk of developing gout, but once they develop diabetes their risk may drop to a lower level than that of normal individuals in the Third National Health and Nutrition Examination Survey [92]**.**

Lower serum uric acid levels was reported in diabetics and higher levels in prediabetics compared with non-diabetic subjects [47].

Fang *et al.* [45] described that the serum uric acid level was higher in diabetic women than in non-diabetic women, although it was not different between diabetic and non-diabetic men, according to the National Health and Nutrition Examination Survey in the USA. On the other hand, it was reported that hyperuricemia was more common in the patients with impaired glucose tolerance than in those with diabetes mellitus or normal subjects. Alderman *et al.* [52] reported that the serum uric acid concentration was not associated with the presence of diabetes mellitus, and Wen *et al.* [49] described that it was negatively correlated with the blood glucose level in a population of 484,568 subjects in Taiwan. Li *et al.* [48] also reported inverse correlations of the blood glucose and

HbA1c levels with the serum uric acid concentration in patients with type 2 diabetes mellitus. Ito *et al.* [86] found, the HbA1c level was also lower in patients with hyperuricemia, and it was negatively correlated with the serum uric acid concentration (HbA1c = -0.17 x uric acid level + 8.33, univariate analysis, P<0.01). Ong *et al.* [82] recently reported that the serum uric acid level did not predict cardiovascular mortality in 1,268 patients with type 2 diabetes in Western Australia.

The discrepancies in the studies are considered to be caused by the effects of insulin on the renal proximal tubules. As a likely mechanism linking HUA and diabetes mellitus, it is known that hyperinsulinemia reduces the urinary excretion of uric acid by activating the transporter of uric acid (URAT), which is expressed in the proximal tubules of the kidney [86].

Pandaru *et al.* observed results of an inverse association between increasing serum uric acid and diabetes mellitus [47].

Recent evidence suggests that uric acid plays a role in cytokine secretion [93]. Further potentially important biological effects of uric acid relate to endothelial dysfunction by inducing antiproliferative effects on endothelium and impairing nitric oxide production and inflammation, e.g., through increased CRP expression, although these issues are considered controversial.

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