

REVIEW

ROLE OF FREE RADICALS IN PATHOGENESIS OF DIABETES NEPHROPATHY

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Abstract

Diabetes mellitus has assumed epidemic proportions in most parts of the world including the developing countries, and one of its ominous complications, diabetes nephropathy represent today the leading cause of end-stage renal disease in the developed countries. However, the pathogenesis of diabetes nephropathy remains illusive, notwithstanding, free radicals seem to be the most favorable linkage between all the associated factors suggested. Consequently, free radicals, oxidative stress and antioxidants have become commonly used terms in modern discussions of renal disease mechanisms, making the kidney unique among other organs as the site in which a spectrum of seemingly unrelated diseases involves reactive oxygen species. Importantly, hyperglycaemia and its attendant metabolic syndromes, smoking and the use of xenobiotics have been shown to accelerate free radical generations and attenuate the antioxidant system creating oxidative stress. The management of diabetes nephropathy is extremely expensive and frustrating. Therefore, prevention is better. Sources of antioxidants, especially antioxidant vitamins are available and affordable in most environments. These may be adjunct to other ways of preventing the development of diabetic nephropathy. Reviews like this are necessary to stimulate stakeholders in management of diabetes mellitus and modern nephrologists.

Key words: Diabetes mellitus, nephropathy, free radicals, pathogenesis

Introduction

McCord and Fridovich opened the field of free radicals and oxidative stress when they discovered an enzyme superoxide dismutase (SOD).¹ Since then reactive oxygen species (ROS) has come to occupy an amazingly central role in a wide variety of diseases. Consequently, free radical, oxidative stress, and antioxidants have become commonly used terms in modern discussions of renal disease mechanisms, making the kidney unique among other organs as the site in which a spectrum of seemingly unrelated diseases involves ROS.²⁻⁵

Diabetes mellitus and one of its complications, diabetic nephropathy, represent a leading cause of end-stage renal diseases (ESRD) in the developed countries especially United States and Europe.^{6,7}

Hyperglycaemia of diabetes mellitus and its attendant metabolic syndromes⁸ - insulin resistance, hyperglycaemia, hypertension, dyslipidaemia, obesity, and some social characters of these patients e.g. smoking and the use of xenobiotics, predispose to diabetic nephropathy. These accelerate free radical generation and attenuate the antioxidant defense system creating oxidative stress^{2,9-13}. Consequently,

increased free radical production and attenuation of antioxidant system is currently receiving the highest attention when discussing diabetic nephropathy. Therefore, stakeholders in management of diabetes mellitus as well as modern nephrologists are recommended to eliminate or prevent the development of the sources of free radicals right from diagnosis of diabetes or even in those with impaired glucose tolerance.

Pathogenesis of diabetic nephropathy

The pathophysiology of diabetic nephropathy can be viewed as a sequence of events evolving in a stepwise pattern as shown in figure 1 and 2, where it starts with endothelial cell dysfunction (ECD) and ends with end-stage renal failure. However, ECD is preceded by glomerular hyperperfusion and hyperfiltration.

The normal endothelial cell

The last 20 years have brought about a lucid realization that far from being only an anatomic barrier to prevent the extravasations of circulating blood into the vessel wall, the endothelium is a

metabolically active system that maintains vascular homeostasis,^{6, 9, 14 - 18} by regulating the homeostatic, inflammatory, and reparative responses to local injuries mediated by NO by inhibiting the activation of an important nuclear transcription factor-Nuclear Factor kappa B (NFkB) that binds to the promoter regions of genes which code for proinflammatory proteins.

Hence, NO production or availability can regulate diverse functions in endothelial cells. Therefore, any event that alters the function of NO will lead to endothelial cell dysfunction (ECD).

Endothelial cell dysfunction (ECD)

As any other organ, the vascular endothelium is subject to dysregulation, dysfunction, insufficiency, and failure. Endothelial cell dysfunction is therefore defined as decreased synthesis, release, and/or activity of endothelial-derived nitric oxide.¹⁰ The pathophysiology of ECD expressed in various degrees is emerging as a hallmark of several highly prevalent renal as well as cardiovascular diseases and other chronic diabetic complications.⁶

What triggers ECD in diabetes mellitus?

A causal relationship between oxidative stress, ECD and diabetic nephropathy has been established¹⁹ by observations that:

- a) High glucose can directly cause ECD and increases oxidative stress in glomerular mesangial cells, a target cell of diabetic nephropathy.
- b) Lipid peroxides and 8-hydroxydeoxyguanosine, indices of oxidative tissue injury, were increased in the kidneys of diabetic rats with albuminuria.
- c) Oxidative stress induces mRNA expression of NFkB genes which in turn promotes production of proinflammatory proteins-TGF-B, fibronectin, laminin, elastin, IL-1, IL-6, and PDGF, and
- d) Inhibition of oxidative stress ameliorates all the manifestation associated with ECD and diabetic nephropathy.

Diabetic nephropathy is preceded by glomerular hyperperfusion and hyper filtration,^{7, 20-22} which occur early in type 1 and in some 15-44% of type 2 diabetic patients at diagnosis, and these play a pathogenetic role in ECD, the early stage of diabetic nephropathy.^{7, 20, 21} The hyperinsulinaemia of diabetes mellitus cause increase reabsorption of glucose and sodium in the proximal convoluted tubule and synthesis of NO via activation of IRS-1, 2. The insulin resistance also diverts more glucose into the pentose phosphate pathway, resulting in increase activation of PKC pathway and increase glomerular prostaglandins production, afferent arteriolar dilatation and efferent arteriolar constriction.^{7, 20 - 23} Hyperglycaemia depresses the synthesis of heparin sulphate proteoglycans with consequent impairment of electrostatic barrier.²⁰ These factors associated with genetic factors, e.g., genetic polymorphism of ACE

and the sodium/lithium counter transport gene, lead to glomerular hyperperfusion and hyperfiltration.²³ The high GFR (about 20-30% increase) in humans has been shown to be strongly associated with nephromegaly.²¹ These factors do not induce such early glomerular events alone but in tandem with oxidative changes.^{7, 20 - 23}

There is little doubt that the primary causal factor for the development of most diabetic complications is prolonged exposure to hyperglycaemia. The mechanisms of damage involve,^{20, 23, 25} nonenzymatic glycation of proteins, flux of glucose through the sorbitol pathway, increased de novo synthesis of diacylglycerol and subsequent activation of the protein kinase C pathway, impairment of electrostatic barriers, and increased lipid peroxidation. These not only generate ROS but also attenuate antioxidant mechanisms creating a state of oxidative stress.

The process of nonenzymatic reaction of glucose with proteins is well known phenomenon and has been proven to account for numerous features of chronic diabetic complications.

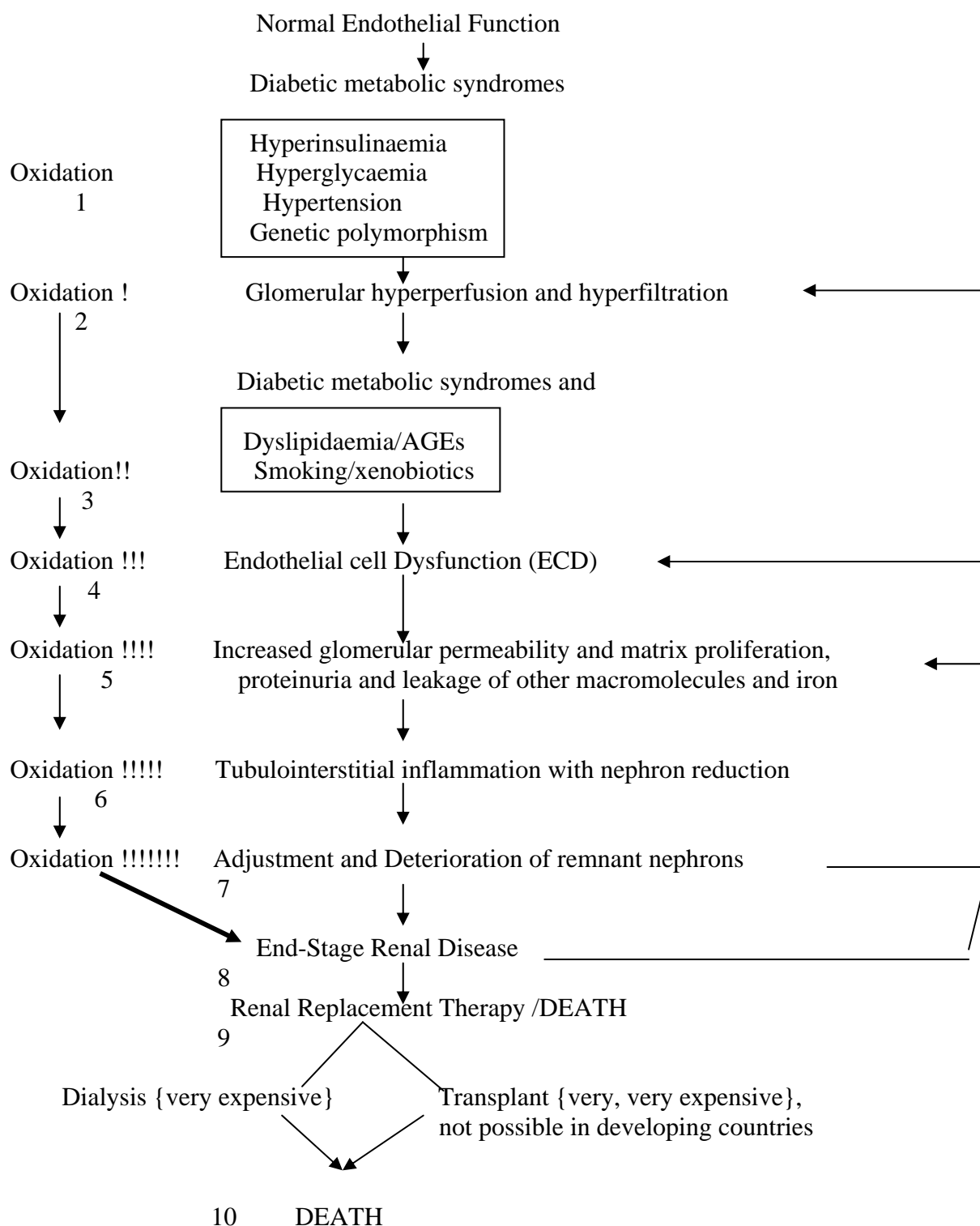
Advance glycosylation end products (AGEs)-receptor specific interactions with endothelial cells and macrophages, binding to antioxidant enzymes, and lipoproteins can lead to widespread ECD.^{6, 20, 26, 27} Lipoprotein trapping and oxidation, deposition of AGEs in glomerular wall, inflammatory cell recruitment and activation, enhanced by clotting abnormalities and platelet activation, follow this. All these events augment production of free radicals.^{6, 26, 27}

There exist in diabetes alteration of blood pressure regulating system especially vascular reactivity as well as volume/sodium homeostasis.^{28, 29} Adherence of diabetic red blood cells to endothelium results in an increase in production of lipid peroxides, and the resulting oxidant stress leads to a marked increase in PECAM-1 phosphorylations and transmigration of monocytic cells. Hyperglycaemia also promotes leukocytes adhesion to the endothelium through up regulation of cell-surface expression of adhesive proteins, and these processes depend on NF-Kb activation. These factors are part of the abnormal blood pressure deregulations.

The glomerular endothelium experiences three primary mechanical forces,³² the transmural pressure, the circumferential stretch (tension) and the shear stress. The altered blood pressure regulation/hypertension alters the shear stress. This appears to result in altered Intercellular Adhesion Molecule (ICAM-1), Vascular Cell Adhesion Molecule (VCAM-1) expression and increase monocyte adhesions, suggesting that the endothelial cells exposed to chronic arterial hypertension may lead to progressive ECD and disrupt the filtration barrier.²⁸ This indicates that oxidative stress is eminent in diabetic hypertensive patients causing ECD and therefore diabetic nephropathy.

Abnormalities in lipid metabolism and the potential nephrotocicity have been suggested more than a century ago,^{30, 31} and these are evidence by.³²

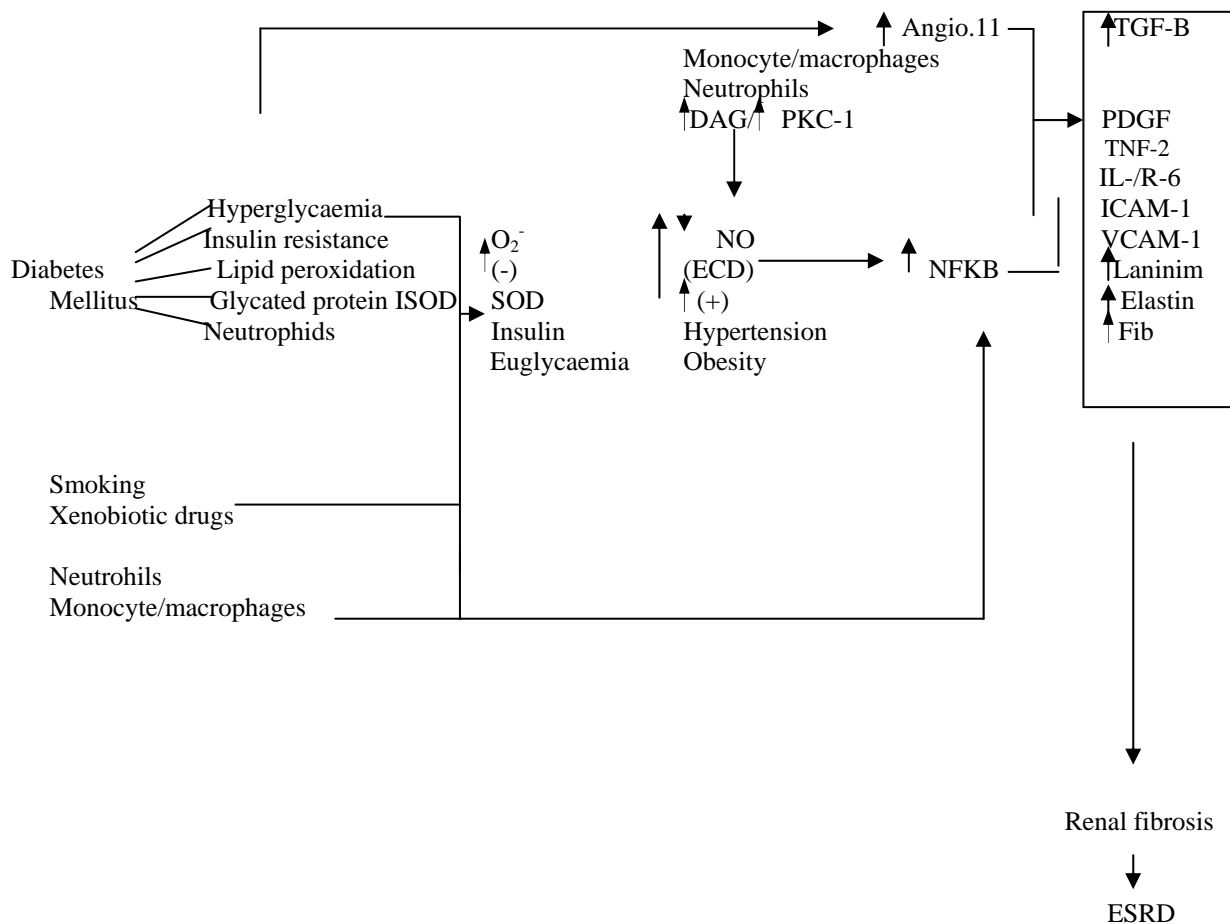
Figure 1: Sequence of events in the pathogenesis of diabetic nephropathy



High cholesterol diets are associated with albuminuria and glomerular injury, lipid lowering agents and intake of large amount of dietary omega-3 and -6 reduced glomerular injury. Diabetes mellitus and renal tissue in this milieu has been suggested to accelerate oxidation of lipids.^{6, 20, 23, 26} Diabetes mellitus as an oxidant milieu enhances free radical generation via;^{6, 19, 33, 34} (a) non-enzymatic

glycosylation of proteins (apolipoproteins, enzymes and receptors) leads to chronically elevated lipids and lipoproteins, (b) increase glucose metabolism through the aldose reductase pathways altering the intracellular redox balance, increase de novo synthesis of diacylglycerol and subsequent activation of PKC pathway, and the attenuation of antioxidant mechanisms create an oxidative stress.

Figure 2: Free radicals and cytokines in the pathogenesis of diabetic nephropathy

**Key**

- Fib: Fibronectin
- O₂: Superoxide
- SOD: Superoxide dismutase
- DAD: Diacylglycerol
- PKC: Protein Kinase C
- Angio-11: Angiotensin-11
- NO: Nitric oxide
- ECD: Endothelial Cell Dysfunction
- TGF- β : Transforming Growth Factor- β
- PDGF: Platelet Derive Growth Factor
- TNF-2: Tumor Necrosis Factor-2
- ESRD: End Stage Renal Disea

These predispose the chronically elevated lipids and lipoproteins to free radical oxidation. It has also been found in diabetic patients that lipid peroxides and 8-hydroxydeoxyguanosine, indexes of oxidative tissue injury were increased in diabetic patients. The oxidized lipids are toxic to tissues especially the vascular endothelium, glomerular mesangial cells, smooth muscle cells and renal tubular epithelium. Under this milieu the major cells of the glomerular wall can carry out lipid oxidation.^{2-4, 20, 25, 26} Oxidized LDL exerts many biological effects that may contribute to the ECD and progression of diabetic nephropathy (table 1).^{11, 32, 34, 35}

Although the above considerable evidence support the notion that lipid abnormalities contribute to renal injury in animal models of endogenous and diet-induced hyperlipidaemia, not all models of hyperlipidaemia develop renal injury.³⁰ This apparent paradoxical relationship between hypercholesterolaemia, atherosclerosis and glomerular injury in these models could be the result of differences in types of plasma lipoprotein.³⁰

Obesity, diabetes mellitus, dyslipidaemia and hypertension are common, interrelated medical problems, and are associated with an increased risk of ECD, vascular as well as glomerular diseases.

Cigarette smoking is shown to impose oxidative stress to the body generally, and this may enhance progression of ECD and therefore diabetic nephropathy.¹³

The use of xenobiotic drugs by diabetic patients may also contribute to the development oxidative stress in the body and the kidney in particular.¹³

Table 1: Oxidized lipids in ECD

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1. Oxidized LDL reduces vascular production of prostacyclins and EDGF (NO) and enhances production of thromboxane A2 and Endothelin-1.
 2. Oxidized is immunogenic. Immune complexes of LDL aggregates are efficiently internalized by macrophages via Fc-receptor. Antibodies to epitopes on oxidized LDL have been demonstrated in patients with diabetes mellitus.
 3. Oxidized lipid alters expression of receptors and change membrane signal transudations.
 4. Oxidized lipid can adversely initiate coagulation system by tissue factor and plasminogen activator inhibitor-1 synthesis.
 5. Through the above mechanisms oxidized LDL induces leukocyte-endothelial cell adhesion and promotes secretion of monocyte chemotactic protein-1 (MCP-1) and macrophage colony-stimulating factor (MCS) by endothelium.
 6. Oxidized lipid is a chemo attractant for monocytes and T-lymphocytes and via MCS; it inhibits macrophage motility in vascular wall.
 7. Oxidized LDL induces macrophages to produce toxic ROS, cytokines, proteases, and growth factors.
 8. Oxidized lipids enhance endothelial cell production of superoxide anions.
 9. Oxidized lipids suppress glomerular epithelial cell sterol synthesis and increase cholesterol ester formation.
 10. Oxidized LDL stimulates expression of several genes in the glomerular wall e.g. NF-Kb and IL-1, and their dependent cytokines and adhesion molecules e.g. VCAM-1, ICAM-1, P-selectin, and Endothelin-1.
 11. Oxidized lipoproteins and lipid hydroperoxides induce apoptosis.
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Tubulointerstitial nephritis

In the past, most nephrologists considered the amount of protein found in the urine, simply as a marker of the severity of renal lesion. Today the results of many studies,^{36,37} indicate that protein filtered through the glomerular capillaries have intrinsic renal toxicity, which together with other independent risk factors, can play a contributory role in the pathogenesis of renal damage.^{7, 38} Plasma proteins reaching the tubular fluid are endocytosed by receptor mediated or constitutional pathway,³⁹ and degraded by lysosomes into their constituent amino acids. However, the reserve capacity for this process is limited and increasing the protein load leads to organelle congestion, lysosomal swelling and rupture eventually exposing renal interstitium to the injurious effect of lysosomal enzymes.^{39,40} These up-regulate many NF-kB dependent or independent genes potentially capable of triggering interstitial reactions,³⁶ and generation of ROS, but in some other instances such a relationship of protein overloading and interstitial inflammation appears less evident. However, a critical review of these studies show that these opinions were essentially based on proteinuria in range of 50-70mg/24hr,⁴¹ and short-term study periods,^{41,42} making it difficult to derive any definitive conclusion. The deleterious effects of glomerular macromolecular traffic and proximal tubular reabsorption on the kidneys are not only limited to the protein component alone. Plasma fatty acids bound to albumin by high affinity are filtered together into the renal tubules and are also endocytosed by PCT. The metabolites of

these fatty acids are chemotatic and toxic to the renal tissues hence promoting interstitial nephritis.⁷

In the setting of tubulointerstitial disease, oxidative stress may directly originate from interstitial inflammatory cells, from pathologic events arising from or related to glomerular damage, or from metabolic adaptations occurring in the surviving nephrons.^{5,43,44} Glomerular disease in diabetes allows leakage of red blood cells, a good source of iron, into the urinary space. Consequently human conditions associated with clinical proteinuria particularly diabetic nephropathy, iron excretion in urine is elevated.³⁴ Iron catalyzes oxidative reaction on the epithelial surface or intracellularly via Fenton reaction, producing highly damaging hydroxyl radicals.¹³

The metabolic burden imposed on remnant nephrons obligates increased oxygen consumption in surviving nephrons, and incurs increased generation of ROS.^{45,46}

Neutrophils and activated macrophages are rich source of ROS. These ROS produced under different mechanisms activate NFkB genes with increased production of NFkB gene-dependent cytokines, which independently or synergistically lead to messangial cell proliferations, extracellular matrix expansion, and renal mass fibrosis.

The ESRD also is good source of free radical generation. It has been found that atherosclerosis is common in patients with end-stage renal disease. This is not merely reflects the high prevalence of the traditional cardiovascular risk factors such diabetes

and hypertension but an independent effect of ESRD on the vasculature.⁴⁷ The association of ROS and atherosclerosis is a well-known phenomenon. ESRD accelerates generation of free radical via the metabolic burden imposed on remnant nephrons obligating increase oxygen consumption, the dyslipidaemia, hyperhomocysteinaemia, and hypertension, which are

commonly associated with the ESRD. This explains the vicious cycle demonstrated in figure 1.

There is therefore a gradual and chronic effacement of nephrons with consequent deterioration of renal functions culminating into renal failure.

Therefore, the role of free radicals in the pathogenesis of ECD and hence diabetic nephropathy are as summarized in table 2.

Table 2: Free radicals in ECD and diabetic nephropathy^{5,9-11,36}

1. Superoxide anions and other free radicals oxidize nitric oxide to metabolites e.g. peroxynitrite, which are potentially harmful to the endothelium.
2. ROS may trigger contraction of the mesangium, afferent and efferent arterioles via PAF, cGMP, thromboxanes, and hence modulation of glomerular profile.
3. ROS trigger CD11/CD18-dependent cell adhesion and can stimulate the synthesis of PAF. PAF is autacoids with potent vasoactive and inflammatory properties and can stimulate mesangial cells to produce more hydrogen peroxide.
4. Hydrogen peroxide is a potent activator of neutrophils and their subsequent adhesion to the endothelial cell surfaces.
5. ROS e.g. superoxide anions, hydroxyl radicals, hydrogen peroxide, peroxynitrite, generated by inflammatory cells in the glomeruli, contribute to lipids peroxidation, forming an accelerated vicious cycle. Hypochlorous acids produced from hydrogen peroxide and halides, e.g. chlorine by myeloperoxidase.
6. Hypochlorous acid reacts with ammonia to form chloramines. Ammonia is abundant in renal cells and urinary space. Chloramines are long-lived oxidants.
7. Oxidants generated via myeloperoxidase-hydrogen peroxide-halide system derived from neutrophils inactivates inhibitors of proteolytic enzymes such as elastase, thus accelerate ECD.
8. Superoxide anions can stimulate the NF- κ B gene and its dependent genes with subsequent production of cell adhesion molecules (VCAM-1 and ICAM-1) and vasoconstrictors e.g. Endothelin-1 and E-selectin. These facilitate cell adhesion to the endothelium.
9. ROS can cause apoptosis of the endothelial cells.

Consequently, oxidative stress is surely an inevitable accompaniment of diabetic nephropathy (see fig. 1 and 2 and tables 1 and 2). Therefore, how can diabetic nephropathy-related oxidative stress be dealt with? Eating a diet rich in fruits and vegetables will ensue adequate levels of antioxidant nutrients in the tissue and help the body to resist disease-related oxidative stress.¹³ Fruits and vegetables are available and affordable even in recourse-poor communities.

Conclusion

An understanding of the homeostatic function of the glomerular endothelium is important for the modern nephrologist. The role of nitric oxide in mediating many of the regulatory properties of the endothelium is now recognized, as well as a growing understanding of how excess free radicals, generated under hyperglycaemia and its associated metabolic syndromes considered to be risk factors for diabetic nephropathy, cause endothelial cell dysfunction with loss of nitric oxide bioavailability.

Because many antioxidant therapies appear capable of improving endothelial cell function and the fact that administration of L-arginine has restored different experimental nephropathies, including

experimental diabetic nephropathy, causal relationship between improved endothelial cell function and diabetic nephropathy can be investigated in humans.

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