REVIEW ARTICLE

DYSLIPIDAEMIA, LIPID OXIDATION, AND FREE RADICALS IN DIABETIC NEPHROPATHY: AN OVERVIEW.

D.S.MSHELIA,* H.U.PINDIGA,+

*Chemical Pathology Department, +Histopathology Department,
University of Maiduguri Teaching Hospital, P.M.B.1414, Maiduguri.

Correspondence to: DR.D.S.Mshelia,
Chemical Pathology Department,
University of Maiduguri Teaching Hospital,
P.M.B.1414, Maiduguri, Borno State, Nigeria.

INTRODUCTION

Diabetes mellitus has assumed an epidemics proportion in most parts of the world including the developing countries, and one of its ominous complications, diabetic nephropathy represents today the leading cause of end-stage renal disease in USA, Europe, and Japan [1,2]. However, there is a paucity of records of the magnitude in Africa and some parts of Asia.

The pathophysiology of diabetic nephropathy can be viewed as a sequence of events evolving in a stepwise pattern starting with endothelial cell dysfunction (ECD) and end in end-stage renal failure [2] (Fig 1).

Majority of diabetic associated metabolic syndromes, especially dyslipidaemia predispose to ECD [3-5], and therefore diabetic nephropathy. Diabetes mellitus is frequently associated with dyslipidaemia evidenced by high prevalence rate that range from 16%-40% [6-8], and chronically elevated level of plasma lipids, low-density lipoprotein in particular, leads to modification of structures, importantly through oxidative processes [9]. Renal tissue particularly in diabetes milieu has been suggested to accelerate oxidation of lipids and lipoproteins [2,10-12].

Lipids, particularly LDL oxidation are initiated by reactive oxygen species (ROS), but the rate of oxidation is usually suppressed in plasma by endogenous antioxidants. However, after ECD the lipid is sequested from the antioxidant milieu mainly in the glomerular wall intima and this accelerates its oxidation. The precise mechanism by which oxidized LDL promotes the development of ECD and diabetic nephropathy lesions remains to be elucidated. However, several lines of evidence suggest that oxidized LDL exerts many biological effects that may contribute to the initiation and progression of diabetic nephropathy. Therefore, oxidized lipids are a key early step in the pathogenesis of diabetic nephropathy [3,13-15].

Importantly, trials of antioxidants in diseases where free radicals play an important role in their pathogenesis reveal satisfactory results [16-19], but results of trials of antioxidants in the management of chronic diabetic complications are inconclusive. Nonetheless, most, if not all, were instituted after a long-standing hyperlipidaemia and therefore oxidative stress, with already developed complications.

This paper provides an overview on the involvement of oxidized lipids, lipoproteins and reactive oxygen species in initiation and progression of diabetic nephropathy. This will stimulate nephrologists to plan instituting the use of antioxidants as preventive measures at the diagnosis of the disease or even in those with impaired glucose tolerance. In addition, the use of lipid lowering agents in diabetics with hyperlipidaemia could become an additional cost-effective adjunct of the anti diabetic regimen.

Sequence of events in the Pathogenesis of Diabetic nephropathy.

KEY

AO - Antioxidants

GSH - R Reduced Glutathione

No - Nitric Oxide

ESRF - End-Stage-Renal-Failure

ECD - Endothelial Cell Dysfunction

SMC - Smooth Muscle Cell

ECA - Endothelial Cell Activation

SGE - Advanced Glycosulated End products

Dyslipidaemia in diabetes mellitus

Diabetes mellitus is associated with high prevalence of dyslipidaemia[6-8]. Hyperlipidaemia is arguably usually a secondary phenomenon. It is unique in that it may initiate s self-perpetuating disease, which may exist either independently or in conjunction with the initiating disease. The usual presentation of dyslipidaemia in diabetes mellitus include the following:-[20-23].

There are several possible mechanisms for the causes of dyslipidaemia in diabetes mellitus[20-23].
One function of insulin in non-diabetics is to maintain the balance between intestinally derived and liver-derived triglyceride-rich lipoproteins. Insulin also normally suppresses fatty acids released from adipose tissue in the postprandial state[20,21]. However, in insulin resistance or lack, these regulatory functions fail with consequent flux of free fatty acids and inappropriate production of very-low density lipoprotein (VLDL) by the liver from these substrates. There is also an increased apoC-111/apoC-11 ratio, which slightly lower the activity of lipoprotein lipase (LPL) (generally lower in NIDDM than in controls) [20-23]. These mechanisms favour hypertriglyceridaemia. Due to this hypertriglyceridaemia and decreased LPL activity, the transfer of surface remnant-redundant phospholipids and apolipoproteins from lipolysis of TG-rich lipoproteins to high-density lipoprotein

(HDL) particles is decreased [22].Therefore, there are fewer surface remnants available to be incorporated into the HDL particle. In addition, due to large TG-rich lipoproteins and prolonged residence time in plasma, there is increased exchange of esterified cholesterol from HDL to TG-rich lipoproteins and of TGs to HDL particles [20].The result is enrichment of the HDL particle core with TGs. The enriched HDL has a faster catabolic rate than normal HDL. The small and dense HDL3 predominates in diabetes at the expense of the larger and cholesterol ester rich HDL2 [20,21].Mature HDL2 is necessary for transport of the essential LPL cofactor, apoC-11to nascent VLDL and chylomicrons (CMs). ApoC-11 is an important LPL cofactor and is presented to it by CMs and VLDL to enable hydrolysis of the TGs that they carry. Without recycling of apoC-11 by HDL2, (as is reduced in diabetes), the action of LPL on these large lipoproteins will be greatly reduced.
LDL size is genetically determined but also influenced by environmental factors such as the diabetic milieu. Small dense LDL particles are therefore one of the components of dyslipidaemia of diabetes mellitus and are more susceptible to oxidation[23].

* Increased TG-rich lipoprotein, especially VLDL.
* Increased LDL/HDL ratio.
* Increased apoC-111/C-11 ratio
* Increased free fatty acids.
* Decreased HDL.
* Normal or slightly elevated LDL.
* Altered composition of TG-rich lipoproteins.

Diabetes mellitus, an oxidant milieu.

Diabetes mellitus milieu enhances free radical generation via the following mechanisms [10,12,24-27]:(1) hyperglycaemia directly increases oxidative stress; (2) non-enzymatic glycosylation of both extracellular and intracellular proteins increases production of ROS; (3) metabolism of glucose via the aldose reductase pathways with change in sorbitol-myoinostol concentrations, alters the intracellular redox balance and enhances

generation of ROS; (4) the increased de novo synthesis of diacylglycerol from glycolytic intermediates and subsequent activation of the protein kinase C (PKC) pathway and its synthesis, increases ROS production from increased oxidase activity; (5) hyperglycaemia not only generates ROS via these processes but also attenuates antioxidant mechanisms creating a state of oxidative stress; (6) lipid peroxides and 8-hydroxydeoxyguanosine, indices of oxidative tissue injury are increased in diabetic patients; and (7) inhibition of oxidative stress ameliorates all the manifestations associated with diabetic nephropathy.

Alterations in lipoprotein compositions, modification by either carbamylation or non enzymatic glycosylation of lipoproteins, enzymes, and receptors, lead to chronically elevated lipids and lipoproteins in diabetic patients. These and the oxidative stress in the diabetic milieu predispose the lipoproteins, particularly LDL and VLDL to modifications of their structures, importantly through oxidative process. Oxidized lipoproteins and free radicals have been found to be toxic to tissues especially the vascular endothelium, glomerular mesangial cells, smooth muscle cells and renal tubular epithelium.

Oxidized lipids and free radicals in the pathogenesis of diabetic nephropathy.

Perhaps the most noteworthy observations concerning the role of oxidative stress in human diseases is the commonality of it. This is derived from the fact that oxidative metabolism is a necessary part of every cells metabolism. If that cell is sick or injured in any way will result in mitochondrail injury, then increased production of ROS is likely to occur [29]. 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 ROS [24,27-29].

The pathophysiology of diabetic nephropathy can be viewed as a sequence of events evolving in a stepwise pattern, starting with endothelial cell dysfunction (ECD) and end with end-stage renal failure (ESRF).

In the last 20 years, knowledge have brought about a lucid realization that far from being only an anatomical barrier to prevent extravasations of circulating blood into the vessel wall, the endothelium is a metabolically active system that maintains vascular homeostasis by: (a) modulating vascular tone; (b) regulating solute transport into cell components of the vessel wall, local cellular growth and extracellular matrix deposition; (c) protecting the vessel from the potentially injurious consequencies of substances and cells circulating in blood; and (d) regulating the haemostatic, inflammatory, and reparative responses to local injury [2,5,30].

It has been found that nitric oxide, through stimulations of soluble guanylyl cyclase and subsequent production of c-GMP, can regulate these diversed functions of the endothelial cell either per se and/or through interaction with circulating formed elements and vascular smooth muscle cells [2,5].

Therefore, any substance that decreased the bioavailability of nitric oxide can lead to endothelial cell dysfunction (ECD). Consequently, ECD is defined as decrease synthesis, release, and/or activity of endothelial-derived nitric oxide (eNO) [4]. As a result, the vessel wall in this condition may promote inflammation, oxidation of lipids and lipoproteins, smooth muscle cell proliferation, extracellular matrix deposition or lysis, accumulation of lipid-rich materials, platelet activation, and glomerular leakage with consequent tubulointerstitial damage. The pathophysiology of ECD characterized by these abnormalities expressed at various degrees is emerging as a hallmark of several highly prevalent renal as well as cardiovascular diseases.

Notwithstanding, the precise mechanism by which oxidized LDL and other free radicals promote the development of ECD and diabetic nephropathy, lesions remain to be elucidated. However, several lines of evidence suggest that oxidized lipids and lipoproteins exert many biological effects that may contribute to the ECD and progression into diabetic nephropathy [3,5,13-15].

Some of the characteristics of oxidized LDL can be seen in table 2 [3,5,18].

Silent features of oxidized lipids and lipoproteins [3,5,18].

Reduction polyunsaturated fatty acids.
Increased lipid peroxides.
Increased content of oxysterols.
In lysolecithin content.
Increased negative charges on apolipoprotein B100.
Fragmentation of apolipoprotein B100.
Reduced uptake by the LDL receptors.
Increased uptake by scavenger receptor mechanism.

Subsequently, a causal relationship between oxidative stress, free radicals and oxidized lipids and diabetic nephropathy has been established [2,3,5,13-15], [31-33], (table 3 and 4). In a similar vein Chen et al., demonstrated that native and oxidized LDL have been found to enhance superoxide anion production from diabetic rat glomeruli and this is attenuated by insulin [32]. This again suggests that oxidized LDL may play an important role in the pathogenesis of diabetic nephropathy through enhanced generation of free radicals. However, the most persuasive evidence of oxidized LDL causing ECD came from the studies with antioxidants such as alpha-tocopherol, probucol, butylated hydroxytoluene, and diphenylphenylenediamines, which were shown to decrease LDL oxidation and progression of atherosclerosis [15,18]. Thus, supplementation with antioxidant nutrients may be a better approach in the prevention of ECD and hence diabetic nephropathy.

Oxidized lipids (LDL) in ECD. [2,3,14.15,32,33]

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

Free radicals in ECD [3-5,29,33,34]

(1) ROS e.g. superoxide anion, hydroxyl radical, hydrogen peroxide, peroxynitrite, generated by inflammatory cells in the glomeruli, contribute to lipids peroxidation, forming an accelerated vicious cycle. Hypochlorous acid is produced from hydrogen peroxide and halides, e.g. chlorine by myeloperoxidase.
(2) Hypochlorus acid reacts with ammonia to form chloramines. Ammonia is abundant in renal cells and urinary space. Chloramines are long-lived oxidants.
(3) Oxidants generated via myeloperoxidase-hydrogen peroxide-halide system derived from neutrophils inactivates inhibitors of proteolytic enzymes such as elastase, thus accelerate ECD.
(4) Hydrogen peroxide is a potent activator of neutrophils and their subsequent adhesion to the endothelial cell surfaces.
(5) ROS trigger CDb11/CD18-dependant cell adhesion and can stimulate the synthesis of PAF. PAF is an autocoid with potent vasoactive and inflammatory properties and can stimulate mesangial cells to produce more hydrogen peroxide.
(6) Ros may trigger contraction of the mesangium, afferent and efferent arterioles via PAF, cGMP, thromboxanes and EDRF, hence modulation of glomerular haemodynamic profile.
(7) Superoxide anion and other free radicals oxidize nitric oxide to metabolites e.g. peroxynitrite, which are potentially harmful to the endothelium.
(8) Superoxide anions can stimulate the NF-kB 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.
(10) ROS can cause apoptosis of the endothelial cells.

Consequences of ECD.

Glomerular injury of any type impairs glomerular permeability leading to leakage of proteins including transferrins and other macromolecules into the urinary space.

In the past, the amount of protein found in the urine, taken as an indicator of the underlying abnormalities in the glomerular permeability, was considered by most nephrologists simply as a marker of the severity of renal lesions.

Today the results of many studies indicate that protein filtered through the glomerular capillary have intrinsic renal toxicity, which together with other independent risk factors such as hypertension can play a pivotal role in the pathogenesis of renal damage.[34-37]

The iron bound to transferrin is released in the tubular lumen and is continuously reabsorbed up to toxic concentration. Iron from transferrin, red blood cells, heamoglobins and other haem-containing proteins in the urinary space, catalyze oxidative reaction on the epithelial surface or intracellular fluid via the Fenton reaction, producing highly damaging hydroxyl radical [38]. Excess iron reabsorbed by the tubules is extruded back into the final urine, hence in human conditions associated with clinical proteinuria, such as diabetic nephropathy, iron excretion in the urine is elevated as well [39].

The deleterious effects of glomerular macromolecular traffic and proximal tubular reabsorption on the kidney are not only linked to the protein component alone. Plasma fatty acids bound to albumin is endocytosed by the proximal convoluted tubules (PCT). The metabolites of fatty acids and lipoproteins are chemotactic to inflammatory cells and hence promote interstitial nephritis [37].

The above mechanisms lead to gradual and progressive reduction of the nephrons.

Excess reactive oxygen species(ROS) may be generated because of metabolic adaptations of surviving nephrons. This pathway is founded on the premise that the nephron in vivo generates hydrogen peroxides normally.

Therefore, through the metabolic burden imposed on remnant nephrons obligate increased oxygen consumption and incurs increased generation of ROS.

The ROS produced in the various mechanisms expressed above activate NF-kB gene with increases production of NF-kB gene-dependent cytokines-IL-1,IL-6.TGF-B,ET-1, TNF-B, and PDGF. These cytokines and growth factors independently or synergistically lead to mesangial cell proliferation, extracellular matrix expansion, and renal mass fibrosis. These will lead to consequent deterioration of renal function culminating to renal failure.

Lipid peroxidation, oxidative stress, and free radicals are probably inevitable accompaniment of diabetic nephropathy.

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 [38]. Fruits and vegetables are available and affordable even in the rural areas of the developing world.

Notwithstanding, in general, trials of antioxidants in the treatment of human diseases have given mixed results to date, except in diseases in which oxidative stress may be causative e.g. Keshan disease.

However, several reasons can account for this: (a) oxidative stress may occur but not important in the pathogenesis of that particular disease; (b) insufficient antioxidant has been used to reach the site at which it is needed or to remove enough radicals. (c) the wrong antioxidant has been used. (d) antioxidant has not been administered for long enough; and (e) antioxidant might have been given after prolonged oxidative stress and overwhelming complications already developed.

Therefore, a well designed clinical trail to test the effectiveness of antioxidants and lipid lowering agents in prevention of diabetic nephropathy is highly recommended.

REFERENCE

(1) Nuhad I. Bryan B., Piotr S., and Eberhard R. Perspective in renal medicine: Renal disease and hypertension in non-insulin dependent diabetes mellitus. Kid. Int. 1999; 55:1-28.

(2) Goligorsky MS., Chen J. and Brodsky S. Endothelial cell dysfunction leading to diabetic nephropathy: Focus on Nitric Oxide. Hypertension 2001; 37:744-748.

(3) Ischwarial J. Evolving lipoprotein risk factor: Lipoprotein (a) and oxidized low-density lipoprotein. Clin. Chem. 1998; 44:1827-11832.

(4) James KL. Endothelium and Acute Coronary Syndrome. Clin Chem. 1998; 44:1799-1808.

(5) Richard OC 111. Role of nitric oxide in cardiovascular disease: Focus on the endothelium. Clin. Chem. 1998; 44:1809-1819.

(6) Akbar AD. Metabolic syndrome is common in Saudi type 2 diabetic patients. Diab. Intern. 2002; 12:47-49.

(7) Nabeel I., Amin J., Mubarak A., Hassan AM., and Samira Al. Dyslipideamia in Qatari patients with non-insulin-dependent diabetes. IDD. 1996; 7:17-20.

(8) Anaja HP., Isah HS., Abdu-Aguye I. And Oburu NA.Lipid profile in diabetic Nigerian: a Zaria update. IDD. 1995; 6:90-93.

(9) Bukachi F., Rana BS., and Henein MY. Lowering cholesterol in patients with coronary artery disease-the role of statins. Africa Health. 2000; 22:16-18.

(10) Wardle EN. How does hyperglycaemia predispose to diabetic nephropathy? QJM. 1996; 89:943-951.

(11) Edward PF and George LK. Vascular dysfunction in diabetes mellitus. Lancet 1997; 350:S1-9-SI-13.

(12) Helen V., Richard B., and Lihare S. Biology of disease: Pathogenic effect of Advanced Glycosylation: Biochemical, Biologic and Clinical Implications for Diabetes and Aging. Lab. Invest. 1994; 70:138-148.

(13) John FM. Lipids and progressive kidney disease. Kid. Int. 1991; 39:S35-S40.

(14) Witztum JL. and Steiberg D. Role of oxidized low-density lipoprotein in atherosclerosis. J. Clin. Invest. 1991; 88:1785-1792.

(15) Sterberg D. Low-density lipoprotein ozidation and its pathological significance. J. Biol. Chem.1997; 272:20963-20966.

(16) Heitzer T., Just H. and Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. Circulation 1996;94:6-9.

(17) Solzbach U., Homig B., Jeserich M., Just H. Vitamin C improves endothelial dysfunction of epicardial coronary arteries in hypertensive patients. Circulation 1997; 96:153-159.

(18) Devaraj S. and Jialal L. Oxidized low-density lipoprotein and atherosclerosis. Int. J. Clin. Lab. Res. 1996; 26:178-184.

(19) Esterbauer H., Gebicki J., Puhl., and Jurgens G. The role of lipid peroxidation and antioxidants in the oxidative proliferation of LDL. Free Radic. Biol. Med. 1992; 13:341- 390.

(20) Frayn KN. Insulin resistance and lipid metabolism. Curr. Opin. Lipidol. 1993; 4:197-204.

(21) Taskinen MR., Lahdenperi S. and Syvanne M. New insights into lipid metabolism in non-insulin-dependent diabetes mellitus. Aun. Med. 1996; 28:335-340.

(22) Mikko S. and Marja-Ritta T. Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus. Lancet 1997; 350: SI 20-SI 30.

(23) Koletsky S. Pathologic findings and laboratory data in a new strain of obese hypertensive rats. Am. J. Pathol. 1975; 80:129-142.

(24) McCord JM. The evolution of free radicals and oxidative stress. Am. J. Med. 2000; 108:652-659.

(25) Catherwood MA., Powell LA., Anderson P., McMaster D., Sharpe pc, and Trimble ER. Glucose-induce oxidative stress in mesangial cells. Kid. Int. 2002; 61:599-608.

(26) Bambolkar S and Sainani GG. Evaluation of oxidative stress in diabetes with or without vascular complications. J. Assoc. Physicians India 1995; 43:10-12.

(27) Baud L. and Ardaillou R. Reactive oxygen species: production and role ion the kidney. Am. J. Physiol. 1986; 25:F765-F776.

(28) Iekuni I., Shigeru K. and Yoshimasa Y. Renal antioxidant enzymes: Their regulation and function. Kid. Int. 1994; 45:1-9.

(29) Karl AN., Michael F. and Thomas HH. The role of oxidants in progressive renal injury. Kid. Int. 1994; 45:S111-S115.

(30) Clines DR., Pollok E., Buck CA., Loscalzo J., Zimmerman GA., McEver RP., et. al., Endothelial cell in physiology and in the pathophysiology of vascular disorder. Blood 1998; 91:3527-3561.

(31) Ha H. and Kin KH. Pathogenesis of diabetic nephropathy: the role of oxidative stress and Protein Kinase C. Diabetes Press. Clin. Pract. 1999; 45:147-151.

(32) William FK., Wayne SM., Bertram LK., Youngki K., and O'Donnell MP. Hyperlipideamia and progressive renal disease. 1991; 39:S41-S48.

(33) Cybulsky MI., Gimbrone MA. Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherosclerosis. Science 1991; 251:788-791.

(34) Remuzzi G. and Bertani T. Is glomerulosclerosis a consequence of altered glomerular permeability to macromolecules? Kid. Int. 1990; 38:384-394.

(35) Remuzzi G. Abnormal protein traffic through the glomerular barrier induces proximal tubular cell dysfunction and qcauses renal injury. Curr. Opin. Nephrol. Hyperten. 1995; 4:339-342.

(36) Eddy AA., Mc Culloch L., Liu E., and Adams J. A relationship between proteinuria and acute tubulointerstitial disease n rats with experimental nephritic syndrome. Am. J. Pathol. 1991; 138:1111-1123.

(37) Giuseppe R., Piero R., and Ariela B. Perspective in clinical nephrology: Understanding the nature of renal disease progression. Kid. Int. 1997; 51:2-15.

(38) Oxidative stress: In: Gutteride JMC and Halliwell B. Antioxidants in Nutrition, Health and Disease, Oxford University Press, Oxford New York, Tokyo 1994:pp90-123.

(39) Howard RL., Buddington B., and Alfrey AL. Urinary albumin excretion, transferring-iron excretion in diabetic patients. Kid. Int. 1991; 40:923-976.