Advanced glycation end-products (AGEs) are a heterogeneous group of compounds that are produced by nonenzymatic, sequential glycation and oxidation of sugars with free amino groups on proteins, peptides, or amino acids. This sequence of events is known as the Maillard reaction (browning), first identified in 1912.1 Initially, aldehyde of glucose molecule produces a labile covalent bond with the amino acid side chain (specifically lysine residues in protein) of both sugar and lipids. This initial glycation reaction is reversible & leads to development of fructose – lysine residues, a Schiff base. This Schiff base, in turn, undergoes molecular re-arrangement to form one ketoamine known as Amadori product.2 Covalent crosslinking of these Amadori products once again with the sugar group in other glycated protein lead to development of irreversibly modified molecules, collectively termed Advanced Glycation End Products (AGEs) (Figure 1).3 Some important examples include the followings:-

- N-carboxymethyllysine (CML),
- Pentosidine and
- Methylglyoxal (MG) - most reactive AGE precursor in endothelial cells.
- Glycated (or glycosylated) haemoglobin A1c (Hb A1c) – helps monitoring glycemic control in diabetic patients.

AGE formation is a natural process that occurs in all individuals. Chronic hyperglycaemia as in diabetes mellitus can accelerate AGE formation in different tissues.4 N-carboxymethyllysine (CML), pentosidine, and Methylglyoxal MG are among some of the well characterized compounds that commonly are used as AGE markers. AGEs can be measured by a variety of techniques including ELISA, HPLC, or mass spectrometry.5 Recently, it has been found that smoking and modern Western diet provides a relatively large...
portion of preformed AGEs (esp CML and MG) and precursors in the form of preservatives or flavouring agents.6

At the cellular level, there are intracellular protective systems which also limit the accumulation of reactive AGE derivatives. The above homeostatic systems, however, can be overwhelmed in high AGE conditions such as diabetes and renal failure, especially when combined with increased dietary AGE intake (Figure 2).7

MECHANISM OF PATHOGENICITY OF AGES: AN OVERVIEW

AGEs induced modified body protein can damage cellular structure and function by three general mechanisms:

i. Modified intracellular proteins behave abnormally;

ii. Modified extracellular matrix components (ECM) interact abnormally with other matrix components and integrins, expressed on cell surface; AGEs cause ECM protein cross linking and abnormal functioning. These AGE induced crosslinks decrease elasticity in arteries and glomerular mesangium and increase permeability.9

iii. Modified plasma proteins bind to receptors for AGE (RAGE) on cells such as monocyte/macrophages thereby inducing the production of ROS (reactive oxygen species), which in turn activates the pleiotropic transcription factor, nuclear factor κB (NFκB), and p21 RAS which in turn alter the expression of several genes, including cytokines (IL – 1, 6, 8; TNF – α), transforming growth factor β (TGF – β), macrophage colony stimulating factor (MCP), VEGF, PDGF, granulocyte – macrophage colony – stimulating factor (GMCSF), insulin - like growth factor I (IGF – 1), thrombomodulin, tissue factors and CAMs (VCAM1 etc) in various cell types.8, 11, 12. However recent studies indicate that mediators other than AGEs may be the major ligand for RAGE.

These include S100/calgranulin family and high mobility group box 1 (HMGB1), all of which are increased by diabetic hyperglycemia.13

Cdc42, Cell division cycle 42 protein; DAG, diacyl glycerol; eNOS, endothelial nitric oxide synthase; LDL, low density lipoprotein; MAP, mitogen-activated protein; NAD(P)H nicotinamide dinucleotide phosphate; NO, nitric oxide; PKC, protein kinase C; ROS, reactive oxygen species.

REACTIVE OXYGEN SPECIES (ROS):

By this time we know that as a result of binding of AGEs with its receptors RAGE on macrophages/ polymorphonuclear leukocytes (PMNs) lead to development of ROS. These are (Figure 3):

- Superoxide anion (O₂⁻) (number 2)
- Hydroxyl radical (OH⁻) (number 4)
- Hydrogen peroxide (H₂O₂) (number 5)
- Hypochlorite ion (OCl⁻)

A radical (also called ‘free radical’) is a cluster of atoms one of which contains an unpaired electron (shown in black star). A radical is very unstable & so very quickly reacts with either a molecule (making it an unstable radical) or one radical to achieve the stable configuration (Figure 4).

When two radicals unite, each contributes its unpaired electron to form a covalent bond.

Stability of two radicals through covalent bond is called crosslinking which is an important step for formation of AGES.

DEFENCE AGAINST ROS:

Oxidative stress results due to an imbalance between ROS and cellular antioxidant defence system. Molecules acting as antioxidant in our body are: –

- Superoxide dismutase – converts two superoxide anions into a molecule of H₂O₂ and O₂
- Catalase
- Alpha-tocopherol – breaks covalent link between fatty acid side chains.
- Uric Acid – perhaps the long life span of some reptiles & birds is attributable to their high levels of uric acid.

Imbalance between ROS & antioxidant may result from: –

- Alteration of glucose metabolism
- Activation or dysregulation of several enzymes not directly involved in glucose metabolism.

Oxidative stress may cause cellular dysfunction by: –

- Promoting formation of AGEs
Advanced Glycation End Products (AGEs): It’s Role in the Pathogenesis of Diabetic Complications

• Inducing DNA strand breaks
• Causing dysfunction of eNOS (endothelial NO synthase)
• Activating p38 & other stress activating pathways leading to apoptosis.

ROLE OF AGES IN THE PATHOGENICITY OF DIABETIC COMPLICATIONS:

Diabetic retinopathy

Many differentiated cells of the eye have little or no regenerative capacity & thereby making them highly susceptible to aging and systemic diseases which accelerate AGE modified macromolecules. A few well defined AGE moieties are associated with retinopathy such as CML, pentosidine or crossline. As with other microvascular environments, AGEs have been localised to retinal vessels and neuroglia of diabetic patients. In particular, sedentary cells resting in direct contact with ECM and/or BM encounter AGE crosslinks. Structural changes observed in diabetic retinopathy are thickening of the capillary BM, enhanced vascular permeability, and loss of pericytes leading to microaneurysm formation. Normally retinal capillaries consist of contractile pericytes & endothelial cells at 1:1 ratio approximately. This ratio drops to 1:10 in moderate to severe stages of nonproliferative retinopathy. These areas of absent pericytes are generally associated with microaneurysm
formation due to lack of support. Accumulation of AGEs in retinal pericytes produce modification of catalase and superoxide dismutase resulting in induction of ROS. Recent evidence also suggests that these adducts can induce osteoblastic differentiation and calcification in pericytes and a potent apoptotic death response from PDGF – BB.

Binding of AGE to its specific receptors (RAGE) in endothelial cells of retinal capillary and retinal hypoxia together increases the expression of a host of angiogenic factors, including, among others, VEGF & placenta growth factor (PIGF), that in turn promote neovascularisation (proliferative diabetic retinopathy).

**Diabetic peripheral neuropathy**

The pathogenesis of the common distal sensory loss is proposed to be multifactorial with its roots tied in pathogenic processes such as nerve hypoxia/ischemia, oxidative stress through ROS, overactivity of polyl pathway, AGEs, deficiency of γ-linolenic acid and protein kinase C (PKC) along with growth factor(s) deficiency, dysimmune mechanisms on the top of genetic predisposition.

Hyprglycemia induced AGEs on peripheral nerve myelin contributes to segmental demyelination by increasing its susceptibility to phagocytosis by macrophages; it also modifies axonal cytoskeletal proteins (tubulin, neurofilament, actin), resulting in axonal atrophy and degeneration with reduced regeneration due to glycation of laminin.

A vicious cycle gradually supervenes; AGE – RAGE interaction producing ROS, ROS accelerating AGE generation, and AGE quenching NO. The quenching action of AGE binding on NO is relevant to nerve ischemia. The reduction of NO is one of the most important mechanisms of ischaemic nerve injury.

AGEs by increasing macrophage recognition and uptake, stimulates macrophage – derived growth factor. This results in proliferation of smooth muscle and atherogenesis. Various experimental designs have demonstrated that there is also an AGE – induced increase in LDLs in vessel walls. AGE binding with RAGE is followed by activation of NF-κB, generation of ROS and an inflammatory response owing to induction of specific DNA binding activity of NFκB in the VCAM-1 promoter region. **Endoneurial NO deficiency** has a number of important consequences in the microvasculature. It normally inhibits the expression of P-selectin, ICAM-1, VCAM-1, and other adhesion molecules, possibly via inhibition of PKC and preventing the activation of NF-κB. NO also inhibits the cytoassembly of NADPH oxidase, thus attenuating ROS release by leukocytes. Therefore AGEs playing the role of an amplifier of the surmounting inflammatory response in the diabetic milieu, brings about a plethora of changes through activation of NF-κB, expression of adhesion molecules, activation of cytokines, inhibiting NO and generation of superoxide anions which finally leads to peripheral neuropathy.

A role for PKC (Protein Kinase C) activation in the microvascular changes in diabetic nerve was demonstrated in an animal model, in which PKCβ inhibitor prevented diabetes induced impairment of nerve blood flow.

**Diabetic nephropathy:**

The hypothesis that AGEs are involved in diabetic kidney disease is based on several observations. In both human and experimental diabetes, CML is identified in mesangial matrix, glomerular basement membranes, tubular basement membranes, and many vessel walls whereas no expression of CML could be detected in normal human glomeruli. Similarly low – level RAGE expression is restricted to podocytes in normal control human glomeruli but glomeruli of patients with diabetic nephropathy demonstrate diffuse upregulation of RAGE expression in podocytes, co-localizing with synaptopodin expression.

AGE mediated ECM protein crosslinking increases rigidity and reduces the susceptibility to enzymatic digestion, thus inducing thickening. This also favours trapping of plasma proteins (LDL and IgG). In addition, AGEs can elicit a variety of cellular responses by binding to RAGE present in mesangial cells and tubular epithelial cells. Interaction of AGE adducts with the RAGE receptors induces the synthesis and release of cytokines, such as TGF-β1, connective tissue growth factor, PDGF, and IGF – 1, and results in enhanced production of collagen, laminin, and fibronectin. RAGE expressed on tubular epithelial cells have also been incriminated in induction of tubular epithelial cell transdifferentiation to myofibroblasts, a key event in the development of tubulo-interstitial fibrosis.

The transcription factor NFkB plays a pivotal role diabetic nephropathy. In vitro studies have demonstrated that AGEs induce NFkB activation via ROS and activation of PKC. NFkB is believed to play a key role in proteinuria induced tubulointerstitial damage, increased production of MCP-1, apoptosis of glomerular epithelial cells, and modulates the TGF-β1 intracellular signaling pathway. Both ACE inhibitors and statins are potent NFkB inhibitors, and their renoprotective action may be related, at least in part, to the suppression of NFkB activity.

PKC is the universal mediator of diabetic microvascular complications. PKC activation regulates vascular permeability, contractility, cellular proliferation, basement membrane synthesis, and signal transduction mechanisms for hormones and cytokines. PKC is a crucial downstream mediator of TGF-β1, angiotensin II and VEGF. Multiple cellular and functional abnormalities in the diabetic kidney have been attributed to the activation of the PKC pathway such as, mesangial proliferation, proteinuria – tubular damage
DIABETIC MACROVASCULAR PATHOLOGY: ATHEROESCLEROSIS

In the vasculature, the principal pathological consequence of AGE interaction with endothelial surface RAGE is the induction of intracellular ROS, linked to the activation of the NAD(P)H oxidase system. \(^{32,33}\) ROS in turn activates the NFXB. Induction of NFXB leads to a transcriptional activation of many genes including TNF-α, IL 1, 6 and 8, IFN-g, and CAMs (E – selectin, ICAM1, VCAM1). \(^{34}\)

Both receptor mediated and receptor independent actions of AGES on endothelium results in loss of barrier function with increase in permeability accompanied by alterations of the physical integrity of the endothelium, as shown by the destruction of organized actin structures and alterations of cellular morphology. \(^{35}\) AGES accumulation on collagen, albumin and apolipoproteins on vascular ECM result in cross – linking of macromolecules. This results in decreased vascular elasticity and reduced vascular compliance with increased stiffness. There is also a reduction of thrombomodulin expression and induction of tissue factor expression in AGE exposed endothelium. \(^{36}\) This changes the dynamic endothelial hemostatic property from an anticoagulant to a procoagulant surface. AGE–RAGE interaction also results in the depletion of cellular antioxidant defence mechanisms such as glutathione and vitamin C and generates ROS. \(^{37}\) AGES linked to the vascular matrix can chemically interfere with the bioavailability of NO, presumably through a direct reaction of the NO radical with ROS formed during the reactions of advanced glycation. \(^{34}\) In parallel, AGES induce the expression of the potent vasoconstrictor endothelin-1 changing endothelial function towards vasoconstriction. \(^{38}\) These prime diabetic vasculature towards enhanced interaction with circulating monocytes.

The interaction of AGES with MPs (macrophages) have been shown to induce a phenotype of activated macrophages, manifested by the induction of PDGF, IGF – 1 and proinflammatory cytokines, such as IL-1 and TNF-a. In MPs, AGE–RAGE interaction prompts chemotaxis. In contrast to the effect of soluble AGES, immobilized AGES, such as those found in BM, slow down MP migration, a process known as ‘apoptaxis’. When MPs reach a site of immobilized AGES in the tissue, their migration is diminished, allowing them to bind to the AGE-modified surface and become activated. This could provide a mechanism for attracting and retaining MPs at sites of AGE deposition in tissues. \(^{34}\)

On the other hand, glycation of apoprotein B and phospholipid components of LDL leads to decreased LDL clearance, increased susceptibility to oxidative damage, and enhanced affinity for scavenger receptor expressed on MPs. \(^{39}\) This results in foam cell formation. The coexpression of AGES, S100/calgranulins, and RAGE, together with dyslipidemia, might contrive the rapid atherosclerosis observed in diabetes. Overlapping distribution of receptors and ligands leads to prolonged cellular activation, paradoxically resulting in increased expression of the receptor. Contrary to other receptors, which are downregulated by increased levels of their ligand, the RAGE–ligand interaction results in a positive feedback loop. \(^{34}\)

Therefore AGE through various interlinked pathways result in vasoconstriction, endothelial dysfunction, MP chemotaxis and apoptosis, increased phagocytosis of lipid laden moieties by MPs and acceleration foam cell formation; all of which culminate in the formation of atherosclerotic plaque. Recently, RAGE overexpression has been shown to be associated with COX–2/PGE1 expression; this effect may contribute to plaque destabilization through the induction of metalloproteinase expression. \(^{40}\)

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