

Diabetic retinopathy– an overview.

Abstract :

Diabetic retinopathy is the leading cause of blindness world over. Neurovascular **degeneration** of the retina is considered presently as the cause as against the vascular changes which are believed to be causative previously. In fact the neuronal changes are shown to ante-date the vascular changes . There is complex interaction of many cells like pericytes, mullers cells ,astrocytes , vascular endothelial cells as well as factors like advanced glycation products, Oxidative and metabolic stress , **inflammatory cytokines** , **leukotrienes** , various growth factors and glycoproteins **etc.** in the pathogenesis of DR . Various biochemical pathways like Polyol pathway, glucosamine pathway, AGE pathway and PKC pathways interacting with one another is also recognised as having a role in the pathogenesis of DR.. But the common link responsible for all these **factors** involvement linking to DM2 is still not clear. The mechanism explaining **Benfotiamine**, a thiamine **analogue** found to be useful in treating DR , though **attractive** in integrating the various biochemical pathways cited above, is not comprehensive. The concept of reactive oxygen species (ROS) is a better alternative **explanations** linking up all pathogenic factors concerned. to chronic hyperglycaemia of DzM2. But **antioxidants** proved futile in treating DR. It may be useful to remember that extensive ROS production in DM2 is consequent to shift of energy metabolism from glycolysis to B-oxidation of fats.. unless this is reversed , ROS production continues . The Diabetes Control and Complications Trial (DCCT) and UKPDS are the two landmark clinical trials that clearly showed the relationship between chronic hyperglycaemia and genesis of DR. progression in T1DM and T2DM patients, respectively. Randomized controlled trials have shown that early treatment of DM2 can reduce an individual's risk of severe visual loss by 57%.intensive glycaemic control appeared long lasting because of the metabolic memory, also known as '**legacy effect**'. A tern which explains the beneficial effects of immediate intensive treatment of hyperglycaemia with a sustained benefit with respect to the outcomes for many years, regardless of glycaemia in the later course of diabetes. Hence the current emphasis is prevention of DR with strict glycaemic control in DM2 ab initio.

Key words :

Diabetic retinopathy(D R) , Advanced Glycation products (AGEs), pericytes , Reactive Oxygen Species (ROS) , Hyperglycaemia.

1.0 Introduction :

Diabetic retinopathy (D R) is an **unique, chronic, complex, progressive , degenerative disease of the neuro-vascular unit** of Retina.

It is **unique because**

a) It is the leading cause of blindness word-wide.

- b) Approximately 6% of diabetics have already evidence of DR when DM2 is first diagnosed.
- c) It is present in 70 % of DM2 pts with duration equal to or greater than 20 years.
- d) 60% of patients with Type 2 diabetes are expected to have some form of retinopathy by the first decade of incidence of diabetes [1]
- e) The changes once started are irreversible though salvage of what is not damaged is possible.

It is **chronic** because the disease insidiously develops over a decade or two, until which it remains asymptomatic. Periodic screening by funduscopy is the only way to detect early retinal changes. It is **complex** because there is no single identifiable factor that alone links hyperglycemia to DR, though multiple factors in the pathogenesis are identified. There is no single target identified for intervention, nor a single factor is attributed to any one particular pathological feature of DR. This implies that Complex interplay of several factors result in DR.

It is **progressive** because, it proceeds from one stage to other relentlessly. To be more precise, DR proceeds clinically from less severe through moderately severe to severe stage of NPDR. From this stage it is computed that in 70 % cases it proceeds to PDR in one year. The ultimate end-point of both types of DRs is loss of vision.

It is a **degenerative** disease because both, neuronal (which includes neurones, photoreceptor cells and supporting cells) as well as vascular degenerative changes mark the underlying pathology of DR. **Neurovascular unit degeneration** is favoured than only the vascular changes believed to be responsible in earlier years, in the pathogenesis of DR. In fact it is now conceded, that neuronal changes anti-date the vascular changes in DR. However which is the cause and which is the effect, still remains a million dollar question.

2.0 DR as a continuous spectrum of events leading ultimately to loss of vision :

Looking from the pathogenesis point of view, both the NPDR (non proliferative) and PDR (proliferative diabetic retinopathy), appear to pursue two different and independent pathways converging on to a single final outcome i.e. blindness. Macular oedema which is the end result of NPDR is preceded by increased capillary perfusion pressure resulting in seepage of fluids and macromolecules from the dysfunctional vascular endothelium (inner retinal barrier) and pigmented neuro-epithelial layer (outer retinal barrier) of retina, limited by its internal limiting membrane (ILM). Whereas, the PDR is preceded by capillary dropout and non-capillary perfusion, leading to hypoxia induced neovascularisation which crosses the ILM and encroaches on to the vitreous and even spreads to iris to involve the trabecular opening causing glaucoma. Even the pathogenic factors operating behind the both conditions seem to be different i.e. cytokines and leukotrienes are implicated in the vascular changes of NPDR whereas growth factors like VEGF etc. are implicated in the genesis of PDR. The other factors like AGEs, oxidative and metabolic stress etc. have been implicated in changes leading to both the types. But there is considerable overlap in the spectrum is supported by the facts that the macular oedema is not the exclusive feature of the initial NPDR but can occur at any stage. When it occurs at a late stage the newly formed vessels themselves are responsible for it. The ischemia of capillaries begins in the NPDR stage itself, especially in the serious type of NPDR. This transitory stage between the NPDR and PDR is known as **Diabetic Macular ischemia** (DMI). Hypoxic ischemia is common to both DMI and PDR and the presence of neovascularisation distinguishes the later. The **micro-aneurysms**, the earliest change in DR are seen in both the types. This earliest stage of DR is called **Background retinopathy**. But the **micro aneurysms of PDR** are formed from **neovascularisation**, and cross the **internal limiting membrane** a differentiating point between DMI and PDR. Further, PDR is never seen de novo and is

always preceded by NDPR. The conceptualisation of both entities as a part of continuous spectrum is advantageous in the sense that detection of earlier (NDPR) changes call for stringent management of hyperglycaemia to keep at bay the late and more serious sequelae of DPR. To this extent the spectrum of events can be viewed as progressing in orderly fashion. Characterised by appearance of microaneurysms which bleed to produce succeeding events consisting of **haemorrhages** and **cotton wool exudates**. The foveal thickness decreases as evidenced by OCT (optical coherence tomography) and avascular zone size increases as seen in FA (Fluorescent angiography). **macular oedema is seen earlier in NPDR** , the severe form of the latter is called **DMI** which is a transitional stage between the two types of DR . From here the progression is to capillary nonperfusion-hypoxia -**neovascularisation** leading to **PDR** .

2 .1 Diabetic Macular Ischemia (DMI)

DMI is defined by an enlargement of the foveal avascular zone (FAZ) and **Para macular** areas of capillary **non-perfusion**. Large proportion (approximately 41%) of patients with DR in a tertiary hospital setting have some evidence of macular ischemia. 2 capillary nonperfusion, capillary drop out and arteriolar narrowing are behind development of **DMI**. that lead to DMI are -

- **Endothelial proliferation,**
- **Platelet and leukocyte adhesion to endothelial wall,**
- **Occlusion and thrombosis of vessel**
and compromised microcirculation and consequent anoxia.

3.0 Role of hyperglycaemia in DR

The **Diabetes Control and Complications Trial (DCCT)** and **United Kingdom Prospective Diabetes Study (UKPDS)** clinical trials confirmed the strong relationship between chronic hyperglycaemia and the development and progression of diabetic retinopathy. Randomized controlled trials have shown that early treatment can reduce an individual's risk of severe visual loss by 57%. [2] The crucial role played by hyperglycaemia gradually unfolds as we proceed, as it is the initiator of diverse pathological events that culminate in producing DR.

The patho-physiology and molecular and biochemical basis of DR

Table - 1

Mechanisms involved in pathogenesis of DR.

- A) Neuronal degeneration.
 - 1) Role of Astrocytes
 - 2) Role of muller cells
 - 3) Role of pericytes.
 - B) Vascular degeneration
 - 1) vascular endothelium dysfunction
 - 2) Blood- Retinal barrier dysfunction
- Role of other factors:
- 3) Inflammation
 - 4) Growth factors.
 - 5) Tight junctions dysfunction.
 - 6) Apoptosis
 - 7) Oxidative stress & ROS.
 - 8) Carbonic anhydrase
 - 9) Role of RASS
 - 10) Role of AGEs

Biochemical pathways :

- 11) Polyol pathway.
- 12) Hexosamine pathway
- 13) AGE PATHWAY.
- 14) PKC pathway.

4.0 Neuro- vascular changes in DR

Diabetic retinopathy is a disease involving neuro-vascular unit, which comprises of retinal vasculature comprising of capillary endothelium, it's supporting structures like pericytes and astrocytes and inner limiting membrane and neuronal epithelium consisting of various types neurons, microglial cells, photoreceptors and outer pigmented layer of retina. Though

the vasculature Changes are thought to be important, neuronal changes are shown to precede them in diabetic retinopathy development. Their cause and effect relationship is debatable.

4.1 Neuronal dysfunction in DR:

neuro retinal function is compromised before the onset of vascular lesions in diabetic.,[3] The retina is a transparent layer of neural tissue between the retinal pigmented epithelium and the vitreous body The retina includes five major cell types that perform sensory, regulatory, nutritional, and immunomodulatory functions. The neurons (photoreceptors, bipolar, horizontal, amacrine, and

ganglion cells) perform sensory functions and define colour perception, spatial resolution, and contrast discrimination. The neuroglial cells sub serve supportive and **nutritional purpose**. Normal vision depends on intact cell-cell communication among the neuronal, glial, microglial, vascular, and pigmented epithelial cells of the retina. Electroretinogram studies suggest that the dim light retinal rod pathway is especially susceptible to diabetic damage.

The macula and peripheral retina operate reciprocally; therefore, the macula is more sensitive than the periphery in bright light, and the converse occurs in dim light

4.2 Role of astrocytes in DR :

- Astrocytes are star shaped glial cell present in retina in addition to brain and spinal cord .
- Glial cells are the interface between the neurons and the vasculature and are thus key regulators of neuronal nutrition and metabolism.
- Vascular leakage occurred with astrocyte loss in early diabetic mice retina as Ang2 increased. The astrocyte loss and vascular leakage were inhibited by intra – vitreal injection of Ang2-neutralizing antibody. In vitro, Ang2 aggravated high glucose-induced astrocyte apoptosis via GSK-3 β activation. Ang2 directly bound to α v β 5 integrin, which was abundant in astrocyte, and the blockade of α v β 5 integrin, in vitro, effectively attenuated Ang2-induced astrocyte apoptosis. In vivo, intra- vitreal injection of anti- α v β 5-integrin antibody inhibited astrocyte loss in early diabetic retinopathy. Taken together, these findings suggest that Ang2 induced astrocyte apoptosis under high glucose via α v β 5-integrin/GSK-3 β / β -catenin pathway.
- The perivascular end feet of astrocyte contribute to the BRB (blood retinal barrier) dysfunction features. Astroglial-endothelial signalling regulates the BRB under pathological conditions.
- In addition, astrocyte is closely related to the formation of tight junction in developing **retina**..[4]
- Retinal astrocytes help to enhance barrier function by releasing soluble proteins. Retinal astrocytes promote the integrity of blood barrier by increasing tight junction protein expression in retinal endothelial cells. [[5]
- kinase anchor protein 12 (AKAP12) is derived by astrocytes. AKAP reduces VEGF level and increases TSP-1 levels leading to enhanced blood barrier through the inhibition of PKC- ζ phosphorylation and Rho kinase activity. [6]

4.3 Role of **Muller cells**

- Muller's cells are neuroglial cells that extend the span of retina
- Müller cells contribute to neuronal dysfunction,
- The production of pro-angiogenic factors like VEGF leading to neovascularization.
- Set up of a chronic inflammatory retinal environment, and eventual cell death. Müller cells produce factors capable of modulating blood flow, vascular permeability, and cell survival,.
- Müller cells recycle neurotransmitters, prevent glutamate toxicity, redistribute ions by spatial buffering, participate in the retinoid cycle, and regulate nutrient supplies by multiple mechanisms.
- Muller glia also contribute to impaired clearance and retinal oedema during diabetic retinopathy.

Portillo et al.'s study suggests that CD40 activation induces Müller cells to release ATP, leading to activation of P2X7 purinergic receptors on retinal microglia and their subsequent expression of inflammatory cytokines. Müller cell-specific CD40 expression is related to the appearance of acellular capillaries in diabetic retina.

4.4 Role of pericytes:

Pericytes are contractile cells that wrap around the endothelial cells that line the capillaries and venules. Their role in DR is summarised below.

- **Micro aneurysm formation:**

Pericyte loss is related to microaneurysms formation [7]. It is possibly due to local weakening and subsequent out-pouching of the weakened vessel walls. Vessels with microaneurysms tended not to contain pericytes, suggesting that their loss permits uncontrolled proliferation of endothelial cells, contributing to micro-aneurysm formation in retinal vessels. 'pericyte dropout' has been used as an index of diabetic retinopathy.

- **Pericyte ghosts formation:**

The selective loss of pericytes from the capillary wall is a histopathological hallmark of diabetic retinopathy, and dropout of these cells is evidenced by the presence of so-called "pericyte ghosts" on histological specimens.

- **Regulation of retinal vascular blood flow :**

Given the potential contractile properties of these cells, it is conceivable that their loss could alter the way blood flow is regulated in the retinal microvasculature. Recently, it is demonstrated that **angiopoietin 2 (Ang2)** induces pericyte apoptosis via **integrin signalling** in DR [16]. Hyperglycemia-induced Ang2 has a central role in pericyte apoptosis by **Ang2/α3β1-integrin/p53** signalling pathway in streptozotocin (STZ)-induced diabetic mice. In addition, Ang2 is upregulated by hyperglycemia.

- **Formation of acellular capillaries, and thickening of the vascular basement membrane.**

[[8]

Consequently, pericyte dysfunction leads to capillary dilation, micro-aneurysms and increased vascular permeability resulting in vascular leakage and macular vascular occlusions in the human diabetic eye

- **Role in PDR:**

In vitro, pericytes control endothelial cell proliferation, suggesting that pericyte loss may be involved in the pathogenesis of proliferative diabetic retinopathy. Loss and degeneration of pericytes affect the integrity and maintenance of BRB.

- **Role in NPDR :** However, pericyte loss also occurs in diabetic rodent models, in which proliferative retinopathy is absent. This suggests that pericytes may also control endothelial function during the initial period of non-proliferative diabetic retinopathy. [9]

Cause of pericyte loss in DR

- Hyperglycaemia and local hypertension are known to be a direct cause of pericyte apoptosis. Dropout is hypothesised due to toxicity due to accumulation of Sorbitol or AGEs. [10]

5.5 Role of Vascular endothelial cells :

- They actively regulate the vascular tone and permeability, the balance between coagulation and fibrinolysis, the composition of the sub endothelial matrix, the extravasation of leukocytes, and the proliferation of vascular smooth muscle. To carry out these functions, the endothelium produces a variety of **regulatory mediators, such as nitric oxide, prostanoids, endothelin, angiotensin II, tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWF), adhesion molecules and cytokines.** Transport of metabolites, fluids, ions and nutrients between blood and the retina is selectively regulated by the physical barrier formed by endothelial cells to maintain homeostasis in the retina.
- Endothelial cells express cell-type specific transmembrane proteins for adhesion including vascular, **endothelial** cadherin (**VE-cadherin**) at **adherens junctions** and **claudins** at tight junctions. [11]
- Transmembrane proteins at tight junctions include **occludin, claudins** and **junctional adhesion molecules** on . [12, 13, 14] **Occludin** associates with **zonula occludens ZO-1, ZO-2 and ZO-3**, which directly bind to filamentous actin (**F-actin**) .
- Tight junctions act as a gate for Paracellular transport, and a fence for maintaining cell polarity.[15]
- At adherens junctions, VE-cadherin is a main component associating with intracellular proteins including **β -catenin, p120, and plakoglobin**. Regulation of VE-cadherin expression and/or phosphorylation can affect the overall endothelial cell junctions and vascular stability through modulation of intracellular signalling pathways.[16]
- Hyperglycaemia affects endothelial cell mitochondria leading to increased ROS level and membrane potential was correlated with the rate of cell death. [17]Hyperglycaemia causes mitochondrial fragmentation with concomitant increases in cytochrome c release leading to apoptosis.[18]
- Hyperglycaemic conditions increase PP2A activity through up-regulation of methylation followed by the activation of apoptotic proteins, superoxide, and NF-**KB**. [19]
- apoptosis of retinal endothelial cells under high glucose is independent of caspase activity. [20]
- Hyperglycemia reduces the expression of connexin 43 and decreases cell-cell communication triggering apoptosis. .[21]
- high glucose conditions disrupted tight junctions by decreasing occludin expression through the activation of VEGF and insulin-like growth factor-I receptor. [22]
- Hyperglycaemic conditions also affect tight junctions by decreasing ZO-1 levels in the retina of diabetic mice.[23] Consequently, such conditions disturb junctional integrity leading to increased retinal endothelial cell permeability.[24]
- High glucose conditions enhanced the migration of retinal endothelial cells which is mediated through activation of Src, PI3K/Akt1/eNOS and ERKs pathways
- [25] as well as through the up-regulation of **heparinase**. The activation of **Akt** and ERK phosphorylation are also involved in the up-regulation of heparinase .[26]
- Increased migration under high glucose conditions contributes to increased angiogenic activity of retinal endothelial cells and is consistent with down-regulation of TSP-1 in microvascular endothelial cells under hyperglycemia and enhanced migration of TSP-1-deficient retinal endothelial cells.[27]

5.0 Blood retinal barrier (BRB) dysfunction.:

RBB consists of Outer and inner BRB and tight junctions.:

5.1 The inner blood-retinal barrier, which is formed by the intra-retinal microvasculature cells surrounded by pericytes and the foot processes of astrocytes. Astro-and **pericytes** are crucial for maintaining the structure and nutrition of inner BRB.

Both the inner and outer RBB are characterised by tight junctions between adjacent cells which effectively limit the passage of solutes, proteins and fluids from the choroidal and intra-retinal blood supplies

Blood constituents may leak into the retina, resulting in **diabetic macular oedema**, the commonest cause of blindness in diabetes. This is the stage of NPDR. When NPDR is severe, ischemic changes set in leading to a pre-PDR, called **diabetic macular atrophy** which progresses to PDR. The latter is due to anoxia induced neovascularisation.

5.2 Outer RBB: Is formed by the pigmented layer of retina. Outer RBB dysfunction leads to increased **permeability** and leak from **chorio-** capillaries contributing to macular oedema.

6.0 Pathology of DR –a bird's eye view.

6.1 Haemodynamic changes

- 1) **Non-proliferative DR.**
- 2) narrowing of the retinal arteries **and reduced** blood flow initially, in short duration of diabetes (less than 5 years)
- 3) Dilation of the retinal arteries and increased retinal blood flow at a later stage.
- 4) retinal perfusion is inversely correlated with levels of glycated haemoglobin in both Type 1 and Type 2 diabetic patients.
- 5) Increased capillary pressure and leakage of macromolecules and fluids
- 6) Macular oedema

6.2 Proliferative diabetic retinopathy (PDR) .

- 1) Capillary **non perfusion and** capillary drop out.
- 2) Hypoxia induced neovascularisation.

Histo-pathological changes in DR :

- ❖ **Basement membrane thickening -**
It is a result of the up regulation of membrane components e.g., **fibronectin, collagen IV and laminin**) and/or **reduced breakdown by proteolytic enzymes.**
- ❖ **"ghost pericytes -**
loss of pericytes- (pockets within the basement membrane that appear to have once contained a pericyte) dysfunction leads to capillary dilation, micro aneurysms and increased vascular permeability resulting in vascular leakage and macular edema.
- ❖ **Acellular capillaries:**
Acellular capillaries are those in which basement membrane or remnant endothelial cell cytoplasm remains while the cell nucleus is absent.
- ❖ **Capillary micro-thrombi, occlusion and capillary drop out**
- ❖ **Arteriolar degeneration.**
- ❖ **Destruction of vascular musculature**
- ❖ **micro aneurysm formation.**

7.0 Tight junction disassembly .

Tight junctions, also known as occluding junctions or zonulae occludens are multiprotein junctional complex whose general function is to prevent leakage of transported solutes and water and seals the Paracellular pathway. Tight junctions may also serve as leaky pathways by forming selective channels for small cations, anions, or water. Interaction with PAR3 and PAR6, PKC ζ also phosphorylates occludin and thereby promotes tight-junction assembly, identifying PKC ζ as a key player in the regulation of tight-junction function integrity. [28] TNF- α signals, through PKC ζ / NF- κ B pathway alter tight junction complexes and increase retinal endothelial cell permeability.[29] Titchenell et al. showed that PKC ζ inhibitor prevent vascular endothelial growth factor (VEGF) induced blood retinal barrier dysfunction. [30] Similar findings were recently reported in the brain where PKC ζ activity mediated hypoxia-induced increase in cortical blood-brain barrier permeability.[31] PKC ζ is probably activated by ceramide pathway.

8.0 Angiopoietins :

Angiopoietins are part of a family of vascular growth factors that play a role in angiogenesis. Angiogenesis, is the process by which new arteries and veins form from pre-existing blood vessels. Angiogenesis proceeds through sprouting, endothelial cell migration, proliferation, and vessel destabilization and stabilization. They are responsible for assembling and disassembling the endothelial lining of blood vessels[[32,33] Angiopoietin cytokines are involved with controlling microvascular permeability, vasodilation, and vasoconstriction by signalling smooth muscle cells surrounding vessels[38]identified growth factors VEGF with additional contributions of other factors like **angiopoietin-1, integrins, and chemokines** play an essential role. VEGF and Ang-1 are involved in endothelial tube formation. There are four angiopoietins: **ANGPT1, ANGPT2, ANGPTL3, ANGPT4**. [34]

8.1 Vascular permeability signalling

Angiopoietin-1 and angiopoietin-2 are modulators of endothelial permeability and barrier function. Endothelial cells secrete angiopoietin-2 for autocrine signalling while pericytes of the extravascular tissue secrete angiopoietin-2 onto endothelial cells for paracrine signalling, which then binds to the extracellular matrix and is stored within the endothelial cells.[35]

Angiopoietin-1 is critical for vessel maturation, adhesion, migration, and survival. Angiopoietin-2, on the other hand, promotes cell death and disrupts vascularization. Yet, when it is in conjunction with vascular endothelial growth factors, or VEGF, it can promote neo-vascularization.

Akt/FKHR pathway in the vasculature is the basis for actions of Ang-1 as a regulator of endothelial cell survival and blood vessel stability. FKHR, regulates many genes associated with vascular destabilization and remodelling (including angiopoietin-2, an Ang-1 antagonist) and endothelial cell apoptosis (e.g., **survivin, TRAIL**). Ang-1 inhibits FKHR-mediated changes in gene expression and FKHR-induced apoptosis.

Ang-1 inhibits, via the **FKHR pathway**, the expression of its antagonist Ang-2. This regulation implies Angiopoietin-2 is produced and stored in Weibel-Palade bodies in endothelial cells and acts as a TEK tyrosine kinase antagonist. As a result, the promotion of endothelial activation, destabilization, and inflammation are promoted. Its role during angiogenesis depends on the presence of **VEGF**. [36] .[41]

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9.0 Role of Apoptosis:

Diabetes-induced cell death has been observed in numerous retinal cell types such as endothelial cells pericytes, neural retinal cells such as ganglion cells, and retinal glial cells such as Müller cells, astrocytes, and microglia.

A study showed that the total number of cell bodies in the retinal ganglion cell layer was reduced by 10% after 7.5 months of diabetes. This was accompanied by a 22% reduction in the thickness of the inner plexiform layer and a 14% loss in the thickness of the inner nuclear layer. In contrast, there was no change in the thickness of the outer nuclear layer, suggesting that more cells were lost from the inner than from the outer retina.

9.1 Effects of Vascular apoptosis :

acellular capillaries: These are capillaries in which basement membrane or remnant endothelial cell cytoplasm remains while the cell nucleus is absent. 1

capillary nonperfusion and retinal ischemia whether is the cause or effect of apoptosis is not known.

9.2 Effect of Neural apoptosis :

Pericyte ghosts; These are due to neuronal apoptosis

Suggested causes of apoptosis:

- glutamate toxicity.
- Loss of trophic support/ signalling.
- Oxidative stress and neuro-inflammation
- **9.3 Types and pathways of apoptosis;** According to Nomenclature Committee on Cell Death (NCCD) there are now 13 subroutines of regulated cell death identified.[37] These include anoikis, autophagic cell death, caspase-dependent intrinsic apoptosis, caspase-independent intrinsic apoptosis, cornification, entosis, extrinsic apoptosis by death receptors, extrinsic apoptosis by dependence receptors, mitotic catastrophe, regulated necrosis, netosis, parthanatosis, and pyroptosis.
- **Extrinsic Apoptosis'** is cell death induced by binding of lethal ligands including FAS/CD95 ligand, tumor necrosis factor α (TNF α), or TNF-related apoptosis inducing ligand (TRAIL) to their respective death receptor. [38]. Upon binding of these ligands to the death receptor, the "death domain" of the receptor recruits the assembly of the "death-inducing signalling complex" (DISC), a platform of various proteins. The DISC results in activation of caspase-8 [[39] active caspase-8 initiates one of two distinct pathways. First, active caspase-8 can directly cleave caspase-3, known as an executioner caspase in the apoptotic process. [40] Alternatively, active caspase-8 can cleave BH3-interacting domain death agonist (BID) creating truncated BID (tBID). tBID then binds Bcl-2 allowing BAX to form a pore in the outer membrane of the mitochondria enabling the release of cytochrome c into the cytosol. This triggers formation of the canonical 'apoptosome' via assembly of APAF1 with pro-caspase-9, cytochrome c, and ATP leading to caspase-9 activation, which in turn activates caspase. [41,42]

Role of inflammation in DR:

10.0 Innate immunity in DR:

Diabetes **induced** inflammation predominantly appears to involve the innate immune system.

Experimental studies have shown that high glucose can induce

1) **monocyte** activation through up regulation of cell surface toll-like receptors

.2) **AGEs** can cause neutrophil activation and dysfunction via the RAGE pathway.

3) monocytes and macrophages express a variety of scavenger receptors, and oxidized LDL may affect monocyte activation through these receptors.

Neutrophil, monocyte and platelet activation has been detected in diabetic patients, which is believed to be responsible for the abnormal leukocyte-endothelial interaction (leukostasis) observed in both patients and animal models of diabetes

10.1 Leukostasis

It is a sign of microvascular chronic inflammation in the early stages of diabetes, and has been postulated to be a factor of retinal endothelial cell death in diabetes

Leukostasis responsible for :

- **capillary occlusion and capillary non-perfusion** seen in diabetic retinopathy.
 - **breakdown of the blood-retinal barrier and increased**
 - **permeability of RBM.**
 - **degenerative vascular pathology in diabetic retinopathy .**
 - **Micro- aneurysm formation**
- The invading pathogens are recognized by pattern recognition receptors, **such** as Toll-like receptors (TLRs) and the receptor of advanced glycation end products [43] The molecules of the pathogens that bind to these receptors are called pathogen-associated molecular patterns (PAMPs) .[44]
- TLR activation or, in absence of pathogens, tissue stress alone, can lead to de-inhibition of the transcription factor Nuclear factor kappa B (NF- κ B), which translocates into the nucleus to stimulate transcription of pro-inflammatory cytokines, acute phase proteins and chemokines, such as IL-6, TNF- α , IL-1 β , and monocyte chemoattractant protein 1. Inflammation normally resolves promptly through a coordinated program involving **resolvins, lipoxins and protectins**. [45] When it is not resolved dramatic consequences ensue

10.2 Extravascular inflammation::Role of microglia and mullers cells :

Microglia and Muller cell activation have been observed in diabetic retina in rats and mice in the absence of clinical pathologies. [46] The activation of microglia in the diabetic retina can lead to the increased production of proinflammatory cytokines, ROS, growth factors, matrix metalloproteinases (MMPs), and nitric oxide all of which have been implicated in the pathogenesis of diabetic retinopathy.

10.3 cytokines :

Inflammatory cytokines—IL-1 β , IL-6, IL-8, TNF- α and MCP-1—have been reported elevated in ocular tissues from non-proliferative DR (NPDR) patients. One study detected even higher levels of IL-8 and TNF- α in diabetic eyes with NPDR than with active PDR. The increase in these cytokines produced by activated microglia, endothelial cells, **microglia**, and later even neurons, highlight the increased activity of these inflammatory cytokines in the early stages of DR and the progression of the inflammatory response throughout all cell types of the retina. The accumulation of these inflammatory mediators has been proposed to contribute to early

neuronal cell death in the retina in DM. Some of the cytokines identified, such as MIP-1, IL-1 and IL-3, are thought to also have a role in angiogenesis, as established in experimental ischemic mouse models suggesting that inflammation also contributes and precedes the development of neovascularization in PDR].Such as IL-8, CCL2, have been detected in the vitreous from patients with proliferative retinopathy Increased

ICAM-1 and LFA-1 expression was observed in human epiretinal membranes removed from PDR patients. Furthermore, CD11a/CD11c+ or HLA-DR+ monocytes and CD3+ T cells were also detected in epiretinal membranes obtained from PDR patients.

10.4 : Significantly higher vitreous levels of neurotrophins (NTs), a group of specific growth factors, such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), NT-3, NT-4, ciliary neurotrophic factor (CNTF) and glial cell line-derived neurotrophic factor (GDNF) could be detected in DR patients, with even higher levels in NPDR compared to PDR [51]. This might be an attempt by glial cells such as MGCs to rescue compromised neurons during this early stage of DR.. **10.9 Oxidised LDL** related to *Pericyte cell death* is known to initiate inflammatory responses and complement activation . [47]

10.5 iNOS:

iNOS is an enzyme that catalyses the reaction for nitric oxide (NO) generation. iNOS is up-regulated in the diabetic retina and plays a crucial role in the pathogenesis of vascular lesion during the early stage of DR. [48]

10.6 Leukotrienes :

by lipoxygenases (LOs), especially 5LO, by leukocytes promotes the retinal capillary degeneration in diabetic mice and contributes to increased levels of pro-inflammatory cytokines. Tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β) are increased in the retinas of diabetic animals.[49]

10.7 Growth factors: In patients with PDR, increased vitreous concentrations of the aforementioned cytokines and neurotrophins along with other growth factors such as VEGF, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-1), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) have been reported . [50]

10.8 Soluble cytokine receptors:

Of note, analysis of vitreous samples from PDR patients also revealed increased levels of soluble cytokine receptors, such as sIL-2R. [51] This increase constitutes a known negative regulatory mechanism of cytokine signalling, suggesting that counter-regulatory mechanisms of angiogenesis and inflammation exist within the eye. note, analysis of vitreous samples from PDR patients also revealed increased levels of soluble cytokine receptors, such as sIL-2R. [51] This increase constitutes a known negative regulatory mechanism of cytokine signalling, suggesting that counter-regulatory mechanisms of angiogenesis and inflammation exist within the eye

10.9 Matrix metalloproteinases:(MMPs)

The development of PDR is a multistage event, including angiogenesis, in which basement membrane degradation, endothelial cell migration and proliferation, followed by capillary tube formation, occur. Matrix metalloproteinases (MMPs), which are important regulators of those migratory and tissue remodelling events, have been reported to be upregulated in PDR as well

[57].**10.8 Role of complement regulators** - expression or impaired function of including **CD55, CD59, and DAF**, has been observed in diabetes as a result of nonenzymatic glycation

10.91 VEGF:

Vascular endothelial growth factor (VEGF) also has a pro-inflammatory role and is elevated in the diabetic retina. The increase in VEGF level promotes neovascularization and damage in the retina . [55]and its inhibition ameliorates vascular leakage in the diabetic retina. [56]

10.92 PEDF:

Pigment epithelium-derived factor (PEDF) is a member of the super family of serine protease inhibitors. It has neuroprotective, antiangiogenic, and **anti-inflammatory** features.[52][57] PEDF levels are attenuated in the diabetic retina and associated with up-regulation of VEGF levels. [53]

10.93 TSP 1 :

lack of TSP-1 and/or its selective down-regulation in the endothelium exacerbates the development and pathogenesis of DR. [54]

11.0 ROS / Oxidative stress and DRP.

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:

Diabetes causes oxidative stress, and **augmented levels** of oxidative stress contributes to the pathogenesis of diabetic vascular complications, including DR . [55] Reactive oxygen species (ROS) is generated from

- 1) mitochondrial electron transport chain,
- 2) cytochrome p 450s,
- 3) the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase
- 4) nitric **oxide** synthases (NOSs). ROS produced by cells is required to maintain normal **cellular functions**. However, excess production of ROS result in pathological conditions **and oxidative** stress

- Mechanism of ROS production

In the diabetic retina, ROS levels are elevated and related with vascular dysfunction, including

- loss of pericytes .
- formation of acellular capillaries.
- vascular leakage
- and thickening of BM. [56]

Oxidative stress in the diabetic retina has causal links with vascular inflammation,

12.0 Metabolic dysfunction due to ROS :

- 1)Vascular endothelial dysfunction.
- 2) RAGE activation, activation of PKCs,
- 3)and activation of nuclear factor kappaB (NF-κB).[57,58,59,60]

Photoreceptor cells have been recently shown to be an early source of oxidative stress and local inflammation in the diabetic retina.

Diabetes increased generation of ROS by photoreceptor cells followed by induction of pro-inflammatory mediators, including inducible nitric oxide synthase (NOS) and intercellular adhesion molecule 1 (ICAM-1). [61]

13 Role of Carbonic Anhydrase (CA) IN D R

- 1) Gao et al. it was shown that diabetic patients had significantly higher concentrations of CA than healthy controls . [62]
- 2) It has been demonstrated that CA inhibitors including acetazolamide and benzolamide can reduce the progression of diabetic retinopathy and prevent visual loss in both animal and clinical studies. [63]
- **The mechanisms suggested are**1)reducing humour secretion, inducing vasodilatation .
- 2)improving blood flow to the ocular region,
- 3) inhibiting platelet aggregation
- 4) And reducing vascular permeability.

14.0 RASS VS DR

- 1) The expression of the receptors and signalling molecules of RAAS, namely, renin, angiotensin converting enzymes I and II (ACEI and ACE II), as well as angiotensin receptors, has been reported to increase in the retina, during PDR.[[64,65]
- 2) Studies on experimental models provide evidence that ACE inhibition prevents neovascularisation, a hallmark feature of early diabetic retinopathy.[66,67] [,71,72] RAAS contributes to diabetic retinopathy through probably angiotensin II involvement in the PKC activation as well as VEGF signalling

5.0 Role of AGEs in D R

Advanced glycation end products (AGEs) are proteins or lipids that become glycated as a result of exposure to sugars. They are believed to play a causative role in the vascular complications of diabetes mellitus . The interaction of AGEs with specific cell surface receptors has been implicated in the development of diabetic retinopathy These AGE receptors include the RAGE, galectin-3, CD36, and the macrophage scavenger receptor .AGEs affect cells by three main mechanisms: (1) as adducts occurring on modified serum proteins, 2) as endogenous adducts formed as a consequence of glucose metabolism, or (3) as extracellular matrix-immobilised modifications of long-lived structural proteins

Production of AGEs :

There are two pathways involved in production of AGEs:

16.1 The Maillard or browning reaction

It begins with the formation of a Schiff base between glucose and ε-amino groups that slowly rearranges to relatively stable Amadori adducts, an example of which is

haemoglobin A1c (HbA_{1c}). Both the Schiff base and the Amadori product can undergo further oxidation and dehydration with concentrations ultimately dependent on both

forward and reverse reactions. The forward reactions give rise to additional irreversible protein-bound compounds collectively termed advanced glycation end products (AGEs). During diabetes, the rate of formation of AGEs exceeds that predicted by first-order kinetics. Thus, over time, even modest hyperglycaemic excursions can result in significant adduct accumulation on long-lived macromolecules.

16.2 The oxidative pathway:

AGEs are produced from DHAP/GD3PH through dioxy glycol pathway .

16.3 AGEs have a range of pathological effects, such as:

- **increased vascular permeability.**
- **increased arterial stiffness Inhibition of vascular dilation by interfering with nitric oxide.**
- **Oxidizing LDL**
- **Binding cells including** macrophage, endothelial, and mesangial—to induce the secretion of a variety of cytokines
- **Enhanced oxidative stress**
- Accumulation of AGE might contribute to increased retinal endothelial cell permeability resulting in vascular leakage .[68] Vessel wall thickening and coagulation, leading to occlusion and ischemia.[69] along with induction of growth factors such as vascular endothelial growth factor (VEGF), result in angiogenesis and neovascularization. Ages can induce crosslinking of collagen which can cause vascular stiffening and entrapment of low-density lipoprotein particles (LDL) in the artery walls. AGEs can also cause glycation of LDL which can promote its oxidation. Oxidized LDL is one of the major factors in the development of atherosclerosis.
- Finally, AGEs can bind to RAGE (receptor for advanced glycation end products) and cause oxidative stress as well as activation of inflammatory pathways in vascular endothelial cells.

16.4 AGE and Muller's cells dysfunction :

Winkler et al. have demonstrated that Müller cells exposed to high glucose conditions in vitro produce excess lactate, indicative of increased glycolytic flux, and this effect leads to increased

16.5 production of MGO.

MC show dysfunction during the hyperglycemia and hypoxia experienced by diabetic retina. This dysfunction is manifested by increased expression of glial fibrillary acidic protein (GFAP), NO production, and concomitant synthesis of glutamate (as a function of disruption of the glutamate transporter which may contribute to excitotoxicity in retinal neurons. 64 Significantly, to greater production of MGO

16.6 AGEs and Pericytes dysfunction :

AGEs induce toxic effects on retinal pericytes by causing oxidative stress and subsequent apoptosis. In addition, some studies have indicated that AGEs cause **osteoplastic** differentiation and calcification in retinal pericytes by the activation of alkaline phosphatases. Pericytes growing on AGE-modified basement membrane show acute attenuation of endothelin-1 (ETA receptor-mediated) contraction, suggesting that AGE cross-linking in a surrounding matrix significantly

influences pericyte physiology. Indeed, longer exposure times to these substrate AGEs induce loss of integrin signalling and apoptosis.

16.7 The role of AGE triggered inflammation:

Galectin-1 is a hypoxia-induced angiogenic factor associated with proliferative DR. Phosphorylation. [69] of VEGFR2 and activating its downstream signal transduction in endothelial cells, promotes hypoxia-induced angiogenesis.[70] together with VEGFR1-mediated vascular hyperpermeability. [71]

Significant up regulation of galectin-1 in eyes of DR patients as disease progressed. AGE stimulation induced the expression of IL-1 β through TLR4 activation and its downstream NF- κ B, ERK1/2 and JNK signal transduction in human monocyte-derived macrophages

16.8 Role of RAGEs(AGE receptors)

AGE receptor binding can initiate important signalling pathways involving

- 1) tyrosine phosphorylation of Janus kinase (JAK)/signal transducers and activators of transcription (STAT). [72]
- 2) recruitment of phosphatidylinositol 3' kinase to Ras[73]
- 3) activation of protein kinase C [74]
- 4) and oxidative stress through NF κ B and AP-1 transcription [75]
- 5) AGEs also inhibit prostacyclin production and stimulate plasminogen activator inhibitor-1 (PAI-1) through an interaction with RAGE. [76]
- 6) AGE-modified serum proteins, such as CML, may interact with vascular endothelium via RAGE, which can activate nuclear factor kappa B (NF- κ B), leading to enhanced expression of adhesion molecules and secretion of cytokines such as tumor necrosis factor alpha (TNF-alpha) and VEGF. Similarly sedentary cells like endothelium encounter AGEs such as pentosidine-derived cross-links within basement membrane proteins where they may disrupt integrin signalling. These findings suggest that AGEs may modify structure and function of target molecules in such a way that they do not respond anymore to biological signals. A schema showing the involvement of AGE-triggered inflammatory cues linking to galectin-1 up regulation in the pathogenesis of DR. AGE accumulation due to prolonged hyperglycemia triggers cellular (macrophage) and molecular (IL-1 β) inflammatory cues linking to glial galectin-1 production. Galectin-1

17.0 Role of AGE intermediaries in causing DR :

17.1 Methylglyoxal (MG) : glycolysis inevitably produces methylglyoxal (MG) as a by-product. The accumulation of the cell-permeant MG is highly deleterious, since this compound is one of the most potent glycation agents produced in cells.

- 1) It readily reacts with proteins, lipids and nucleic acids to form

advanced glycation end products (AGEs)

- 2) Methylglyoxal greatly enhances apoptosis caused by agents that induce oxidative stress and DNA damage.

- 3) increased modification of vascular basement membrane type IV collagen by methylglyoxal, at hot spot modification sites in the RGD and GFOGER integrin-binding sites of collagen, causes endothelial cell detachment and inhibition of angiogenesis

- 4) In addition, matrix glycation impairs agonist-induced Ca^{2+} increases, and this may adversely affect regulatory functions of the endothelium.
- 5) Aminoacetone also rapidly enolizes and thus readily autoxidizes catalysed by transition metals to form enolyl radicals, ROS, and iminoacetone. The latter hydrolyses to form methylglyoxal and NH_4^+ .
- 6) Aminoacetone also reduces and releases Fe^{2+} from mitochondrial ferritin thereby causing mitochondrial oxidative stress.
- 7) Mitochondrial succinate-induced respiration was also inhibited and calcium-dependent mitochondrial membrane peremobilization and swelling occurred. DNA oxidation also occurred. Aminoacetone accumulated in diabetes

17.2 .3 Deoxyglucose (3 DG)

-**Deoxyglucose** (3DG) is a dicarbonyl sugar that is synthesized through the Maillard reaction, [77, 82] and is metabolized to 3-deoxyfructose and 2-keto-3-deoxygluconic acid. 3DG is a precursor for the formation of advanced glycation end-products (AGEs)

Diabetic humans have elevated levels of 3DG and 3-deoxyfructose (3DF) in plasma and urine as compared with non-diabetic individuals. Development of diabetic complications is accelerated in patients with extremely high levels of 3DG in their serum.

3DG plays a central role in the development of diabetic complications via FN3K action.

3DG induces reactive oxygen species (ROS) that contribute to the development of diabetic complications. [78]

3DG inactivates some of the most important enzymes that protect cells from ROS. For example, glutathione peroxidase, a central antioxidant enzyme that uses glutathione to remove ROS, and glutathione reductase, which regenerates glutathione, are both inactivated by 3DG. [79, 84] 3DG induces apoptosis in macrophage-derived cell lines. Accumulation of 3 DG lead to neurotoxicity because of ROS generation. 80. [85] 3DG glycosylates and crosslinks proteins leading to a complex mixture of compounds called advanced glycation end-products (AGEs). [81, 86] contribute to the diabetic complications including retinopathy. 82 [87] Aminoacetone as a source of ROS, cytotoxicity, and Genotoxicity has been reviewed recently (Bechara et al. 2007).

18.0 Biochemical pathways involved in DR:

At least 4 biochemical pathways are involved in DR.

- ❖ The Polyol pathway,
- ❖ The Hexosamine pathway,
- ❖ The Diacylglycerol -PKC pathway and the
- ❖ The AGE pathway, have already been mentioned above.

19.0 Integration of the above pathways into single operating mechanism in DM2 : (Fig)

19.1 ROS as a single unifying factor behind operation of the pathways causing DR:

It is well known that ROS is generated in DM2. ROS may activate aldose reductase, induce diacylglycerol, activate PKC, induce advanced glycation end product formation, and activate the pleiotropic transcription factor nuclear factor-kappa B (NF-kappaB). These data demonstrate that a single unifying mechanism of induction, increased production of ROS, serves as a causal link between elevated glucose and each of the three major pathways responsible for diabetic damage. How the 4 pathways cause DR. The nuclear level, these pathways increase histone acetylation through p300 and activate transcription factors upregulating gene expression of vasoactive factors and extracellular matrix (ECM) proteins. 5,6. These molecules include, endothelin-1 (ET-1), vascular endothelial growth

factor (VEGF), fibronectin (FN) and its splice variant extra domain B containing FN (EDB+FN), collagen etc^{5,6}. It is of further interest that there are regulatory relationships among these vasoactive factors and ECM proteins^{7,8}. Among these, ECM protein EDB+FN is absent in normal mature adult tissues, but is expressed in diabetes.

19.2 Interpretation of mechanism of action of Benfotiamine:

Benfotiamine is a synthetic lipid soluble analog of thiamine (vitaminB1) which is shown to suppress all these pathways , an unified mechanism was suggested as early as 2003. By Inhibition of AR and stimulation of Transketolase enzyme , Benfotiamine acted inhibiting the production of sorbitol by polyol pathway and increased the glycolytic flux through Transketolase, the key enzyme of HMP shunt and thereby increasing the products- F6P and G3P and consequently increasing the flux through glycolytic pathway .The later happening is doubtful as the very end products of HMP pathway can initiate these pathogenic pathways .

Aldose reductase (AR), the first and rate-limiting enzyme in the polyol pathway reduces glucose to sorbitol using NADPH as a cofactor; sorbitol is then metabolized to fructose by sorbitol dehydrogenase, (SDH) which uses NAD⁺ as a cofactor

In normal physiological conditions, the affinity of hexokinase to glucose is high and hence the amount of sorbitol formed is low. But under conditions of hyperglycaemia, AR is highly active and sorbitol produced is considerable.

20.1 increased polyol pathway flux may act through

- decreased (Na⁺&K⁺) ATPase activity,
- sorbitol-induced osmotic stress,
- decrease in cytosolic NADPH,
- and increase in cytosolic NADH/NAD⁺

20.2 Role of polyol pathway in DR

- 1 Sorbitol does not easily diffuse through cell membranes; as a result, it accumulates and causes osmotic damage . [83]
20.3 fructose produced by the polyol pathway can be phosphorylated to fructose-3-phosphate which in turn can be degraded to 3-deoxyglucosone, both of which are s
- glycating agents and can result in the production of AGEs . [84]
- The use of NADPH as a cofactor in the polyol pathway results in less NADPH availability for use by glutathione reductase, which is crucial for the generation of reduced glutathione. This decrease in the reduced glutathione available results in a diminished protective response against oxidative stress [85]
- The aberrant shift of the NADH/NAD⁺ ratio by SDH has been proposed to trigger NADH oxidase which can lead to the increased production of reactive oxygen species (ROS) within the cell. [86]

But therapeutic agents tried against AR yielded mixed results. The long-term sorbinil trial.. [87]an SDI, indicated that sorbinil did not prevent the worsening of the disease except for a slower progression rate.

The Hexosamine pathway:

the HSP is a relatively minor branch of the glycolytic pathway, encompassing ~ 3% of total glucose utilized .Increased flux through HBP is required and sufficient for some of the metabolic effects of sustained , increased glucose flux, which promotes the complications of diabetes, *e.g.* diminished expression of sarcoplasmic reticulum Ca^{2+} - ATP-ase in **cardio myocytes** and induction of TGF- β and plasminogen activator inhibitor –1 in vascular smooth muscle cells, mesangial cells and aortic endothelial cells. The mechanism was consistent with enhanced O-GlcNAcylation of certain transcription factors. Creased flux of glucose via the Hexosamine pathway has been implicated in insulin resistance, diabetic vascular complications (Figure 2), and stimulation of the synthesis of growth factors increased glucose flux by the Hexosamine pathway may direct retinal neurons to undergo apoptosis in a bimodal fashion, that is, via inductionpossibly by altered glycosylation of proteins and via perturbation of the neuroprotective effect of insulin mediated by Akt.

20.4 The AGE pathway :

Molecular basis of AGE induced DR :

- 1)AGE stimulation induced the expression of IL-1 β through TLR4 activation and its downstream NF- κ B, ERK1/2 and JNK signal transduction in human monocyte-derived macrophages
- 2) IL-1 β acting on retinal Müller glial cells enhanced galectin-1LGALS1 expression via IL1R1 together with intracellular **signalling** pathways ERK1/2 and PI3K
- 3) galectin-1 interacts with the N-glycans of VEGFR2, to promote hypoxia-induced angiogenesis **leading to** PDR.
- ,4) Galactun-1, together with VEGFR1- mediates vascular hyperpermeability16. leading to DRM/ NPDR.

20.5 The PKC- DAG pathway:

Synthesis of diacylglycerol begins with glycerol-3-phosphate, which is derived primarily from dihydroxyacetone phosphate, a product of glycolysis (usually in the cytoplasm of liver or adipose tissue cells). Glycerol-3-phosphate is first acylated with acyl-coenzyme A (acyl-CoA) to form lysophosphatidic acid, which is then acylated with another molecule of acyl-CoA to yield phosphatidic acid. Phosphatidic acid is then de-phosphorylated to form diacylglycerol of the microvascular changes in DR are thought to be due to hyperglycemia-induced activation of protein kinase C (PKC)

- 1) The activities of the classical isoforms (PKC- α , - β 1/2, and PKC- δ) are greatly enhanced by DAG and have been linked to vascular dysfunctions and pathogenesis of DR([88.) [93] the plasma membrane.. [89]
- 2) PKC activation can lead to mitogen-activated protein kinase (MAPK) activation and phosphorylation of several important transcription factors that increase gene expressions of various stress related genes like c-Jun kinases and heat shock proteins . [90. 95])PKC-b has been shown to have a role in the form of a signalling component for VEGF and a regulator of

endothelial cell permeability.[91]

4)PKC activation contributes to the overexpression of plasminogen activator-1 (PAI-1),

the **activation** of NADPH oxidase, and the activation of NFκB in many vascular cells including endothelial cells, smooth muscle cells, pericytes, mesangial cells, and others. [92]

20.6 PKC Vs AGE;

increased AGEs in retinal vascular endothelial cells contribute to pericyte destruction (Yamagishi et al., 1999), breakdown of the blood-retina barrier and release of PKC (Stitt et al.,1997), providing an important link between the PKC and AGE pathways in the development of 21.Oretinopathy, and further demonstrating the complexity of these biochemical interactions.

21.0 acyl glycerol and IR :

Activation of PKC-θ by diacylglycerol may cause insulin resistance in muscle by decreasing IRS1-associated PI3K activity.[93] Similarly, activation of PKCε by **diacylglycerol** may cause insulin resistance in the liver . [94]

22.0 Conclusion:

The complexity of pathogenesis of DR is unveiled. Hyperglycaemia is the ultimate factor inciting each of the factors and pathways involved, individually and **collectively**. The interventions **individually** aimed at each of the above mechanisms involved yielded only limited **success**. A cure for D R is still elusive. The Diabetes Control and Complications Trial (DCCT) and UKPDS were the two landmark clinical trials that showed tight glycaemic control [HbA1c value of 7% or less] could reduce risk of DR development and progression in T1DM and T2DM patients, **respectively**. **Randomized** controlled trials have shown that early treatment can reduce an individual's risk of severe visual loss by 57%.i(intensive glycaemic control) appeared long lasting because of metabolic **memory, also** known as '**legacy effect**'. It is a term used to describe the beneficial effects of immediate intensive **treatment** of hyperglycaemia with a sustained benefit with respect to the outcomes for many years, regardless of glycaemia in the later course of diabetes.

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