كلية الصيدلة – جامعة بغداد
وحدة الشؤون العلمية

Oxidative Stress Biomarkers in Diabetic Retinopathy

طالبة الدكتوراه
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فرع العلوم المختبرية السريرية

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الساعة التاسعة والنصف صباحا

قاعة الدراسات العليا / فرع العلوم المختبرية السريرية

Objectives

- To Explain the role of oxidative stress in the development of diabetic retinopathy
- To clarify the possibility of using antioxidants to reduce the risk of vision loss in diabetic retinopathy

Diabetic retinopathy

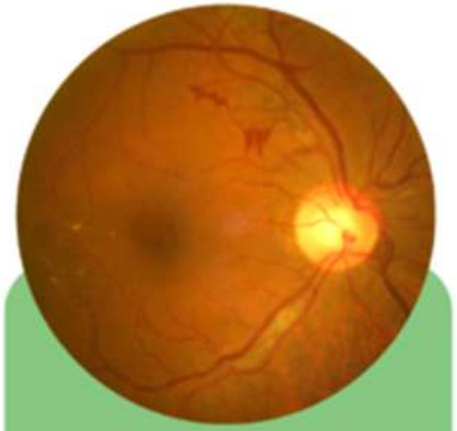
Diabetic retinopathy (DR) is categorized as one microvasculature complication in diabetes, result from an impairment of the inner blood–retinal barrier and microvascular occlusion .

According to the Global Burden of Disease Study, DR is the fifth most common cause of blindness and moderate to severe vision impairment in persons aged 50 and over.

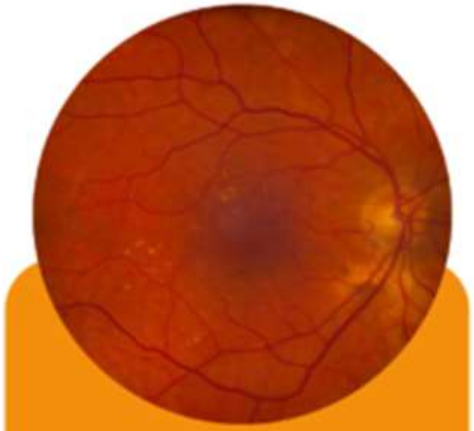
The prevalence rate of DR in Iraqi type 2 diabetic patients from different diabetes mellitus centers.

Sources	Populations With T2DM	Prevalence rate of DR	Centers
[19] Mohammed Al Ashoor *et al* .2.23.	1542	30.5%	Faiha Diabetes, Endocrine, and Metabolism Center (FDEMC) in Basrah, Iraq
[20] Saa'd Bunyan W *et al.* 2022.	272	55.5%	Diabetes center in Thiqar,Iraq .
[21] Raad S. Albayati *et al.*2021.	750	15.3%	Baba Gurgur Diabetic Center in the Kirkuk region of Iraq.
[22] Mohammed DM *et al* .2020.	3026	8.6%	Sulaimani Diabetic and Endocrine Center, Iraq.
[23] Hussain Ali Tufaili *et al.* 2018.	295	27%	Al-Hindea general hospital, Karbala, Iraq
[24] Ala S Tawfeeq *et al* .2015.	289	33.1%	National Center of Diabetes of Al-Mustansiriyah University in Baghdad, Iraq.

Diabetic Retinopathy Classification

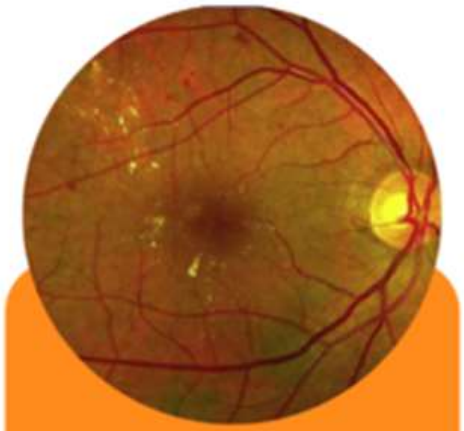


No disease visible



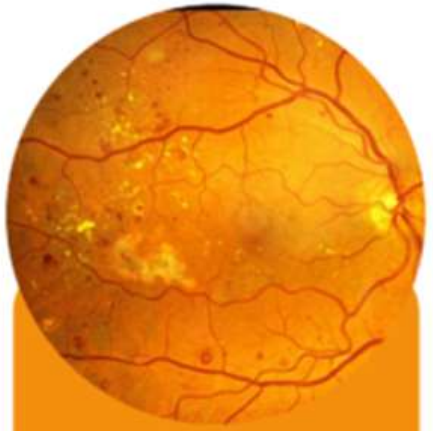
Mild nonproliferative diabetic retinopathy (NPDR)

Localized swelling of the small blood vessels in the retina (microaneurysms)



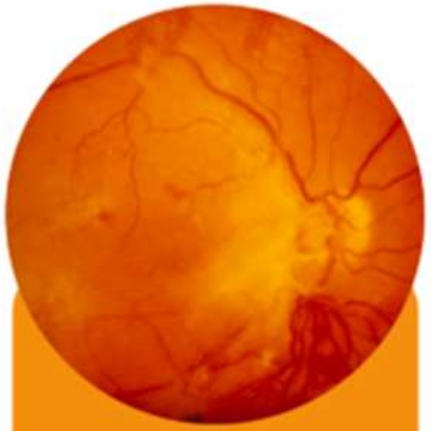
Moderate NPDR

Mild NPDR plus small bleeds (dot and blot haemorrhages), leaks (hard exudates) or closure (cotton wool spots) of small blood vessels.



Severe NPDR

Moderate NPDR plus further damage to blood vessels (interetinal hemorrhages, venous beading, intraretinal microvascular abnormalities).



PDR

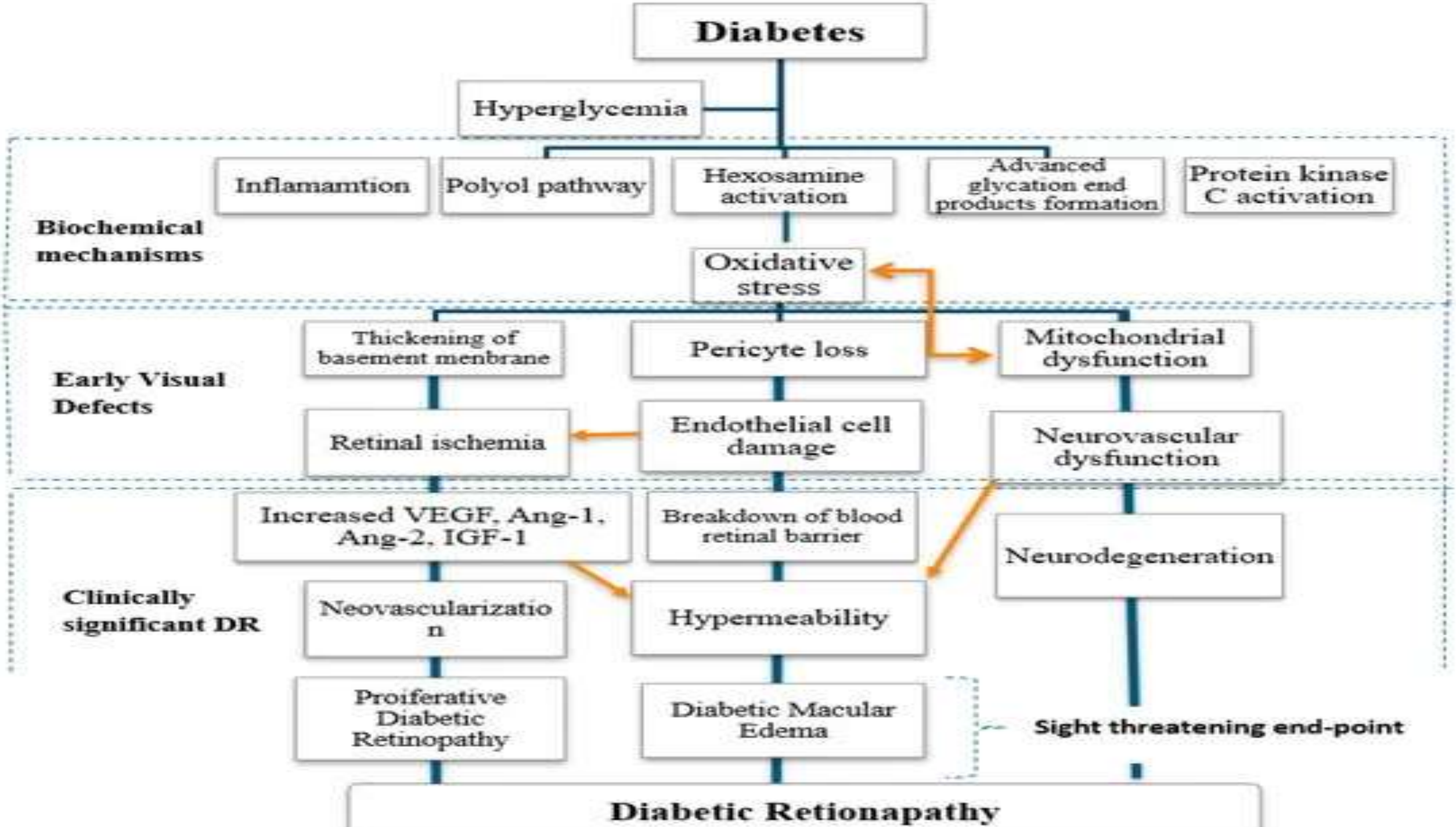
New vessel formation or vitreous/preretina l hemorrhage or tractional retinal detachment

Pathophysiology

- The human eye is constantly subject to oxidative stress(OS), due to frequent exposure to light, in addition to high metabolic activity and oxygen tension.
- Oxidative stress plays a critical role in the pathogenesis of DR. The excessive accumulation of ROS can impair the tissue in and around retinal vessels, eventually leading to DR
- In diabetic, increased glucose flux through glycolysis, results in glycolytic overload, which leads to an abnormal increase in the concentrations of glycolytic intermediates that can be shunted into different damaging pathways such as polyol, hexosamine, protein kinase C (PKC) pathways, and formation of advance glycation end products (AGEs)
- Hyperglycemia induced oxidative damages in the retina by activation of these damaging pathways leads to an increase in oxidative stress either by the generation of ROS or by decreasing the level of antioxidants

- The abnormal activity of nuclear factors, including highly activated nuclear factor-κB (NF-κB) and attenuated activity of nuclear factor erythroid 2 related factor 2 (NFE2L2,) a master transcription factor that controls the transcription of antioxidant enzymes through antioxidant response elements (AREs), and hyperglycemia-mediated mitochondrial dysfunction , have also been demonstrated to be correlated with the overproduction of ROS in DR
- Oxidative stress is responsible for the emergence of various initial clinical indicators of DR, such as the thickening of the basement membrane, apoptosis of pericytes, and dysfunction of mitochondria. These factors collectively contribute to the breakdown of the blood-retinal barrier (BRB)
- The loss of pericytes results in damage to the endothelium. This damage leads to reduced blood flow, known as hypoperfusion, which in turn induces the formation of new blood vessels (neovascularization). Ultimately, this process compromises the integrity of the blood-retinal barrier (BRB)

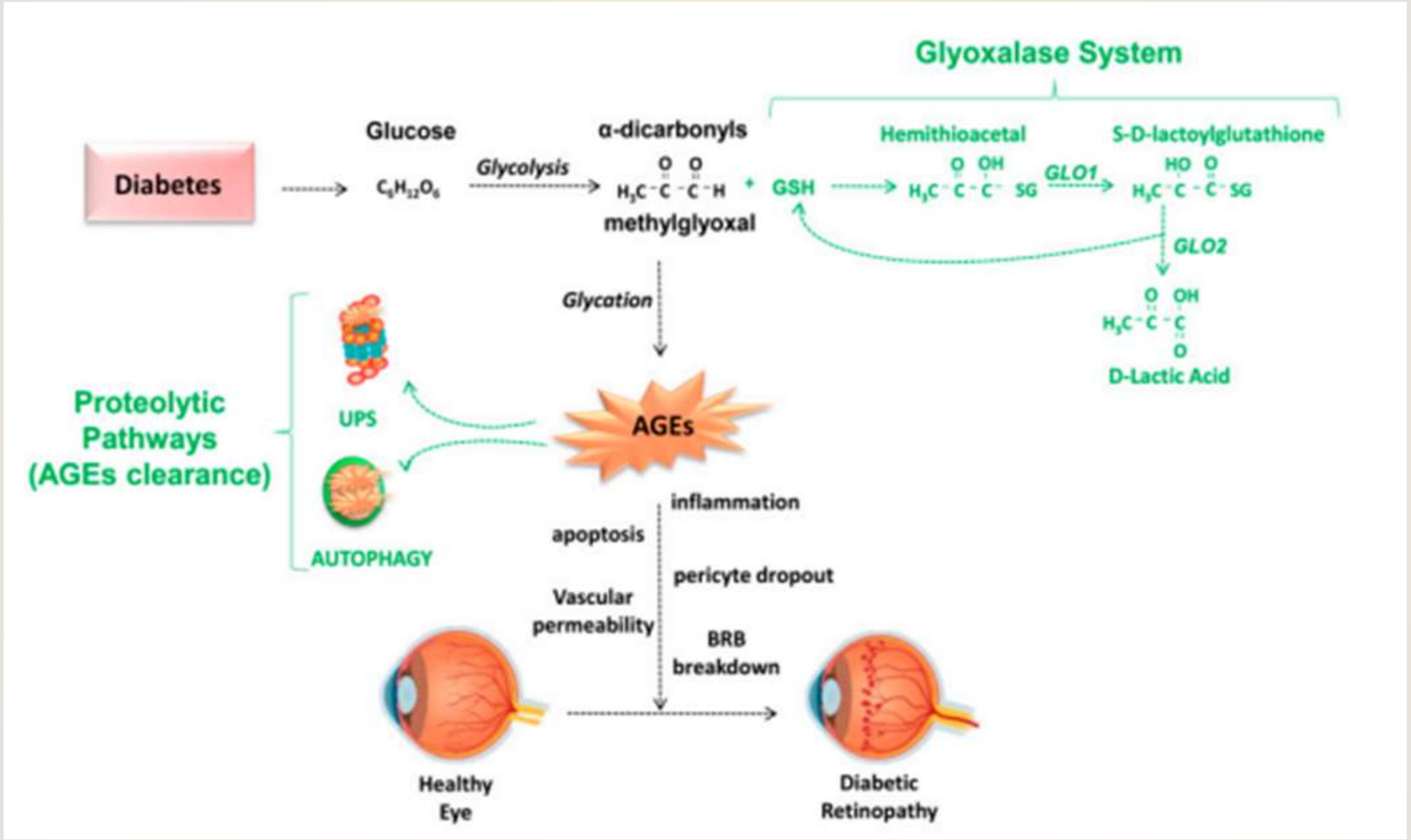
- Furthermore, the depletion of pericytes and the impairment of the endothelium result in the blockage of capillaries and the occurrence of local ischemia. This, in turn, triggers the activation of hypoxia-inducible factor 1 (HIF-1). The activation of this factor subsequently leads to a further increase in the expression of the vascular endothelial growth factor (VEGF).
- Neovascularization generates delicate and permeable blood vessels that are conducive in vitreous hemorrhage. The repetition of such hemorrhages leads to the formation of fibrovascular scars, the contraction of which induce sight-threatening endpoints, namely, PDR


Diabetes
Hyperglycemia
Biochemical mechanisms
Inflamamtion
Polyol pathway
Hexosamine activation
Advanced glycation end products formation
Protein kinase C activation
Oxidative stress
Early Visual Defects
Thickening of basement menbrane
Pericyte loss
Mitochondrial dysfunction
Retinal ischemia
Endothelial cell damage
Neurovascular dysfunction
Clinically significant DR
Increased VEGF, Ang-1, Ang-2, IGF-1
Breakdown of blood retinal barrier
Neurodegeneration
Neovascularizatio n
Hypermeability
Proiferative Diabetic Retinopathy
Diabetic Macular Edema
Sight threatening end-point
Diabetic Retionapathy

Formation of Advanced Glycation End Products

- The advanced glycation end products (AGEs) are a diverse group of reactive molecules, that are formed endogenously by non-enzymatic reactions of carbonyl group of carbohydrates with free amino groups of proteins, nucleic acids or lipids.
- The major glycating biologic reagent is Methylglyoxal (MG), formed by the degradation of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, both glycolytic metabolites.
- Chronic exposure of the retina to high blood glucose levels gives rise to the accelerated formation of AGEs, including CEL, CML, and pentosidine. The increased concentrations of CML and pentosidine in diabetes provides indirect evidence for a diabetes-related increase in oxidative damage to the protein .
- MG is characterized by its cytotoxic properties and high reactivity towards DNA, RNA, and proteins. Glyoxalase I and II play a crucial role in the detoxification of MG under normal physiological conditions .

- The accumulation of AGEs can lead to the programmed cell death of pericytes.
- The viability of retinal pigment epithelial cells is reduced due to the inactivation of glyoxalase I and the subsequent accumulation of MG. This reduction in viability is mediated by the induction of endoplasmic reticulum stress, leading to the production of ROS and dysfunction of the mitochondria

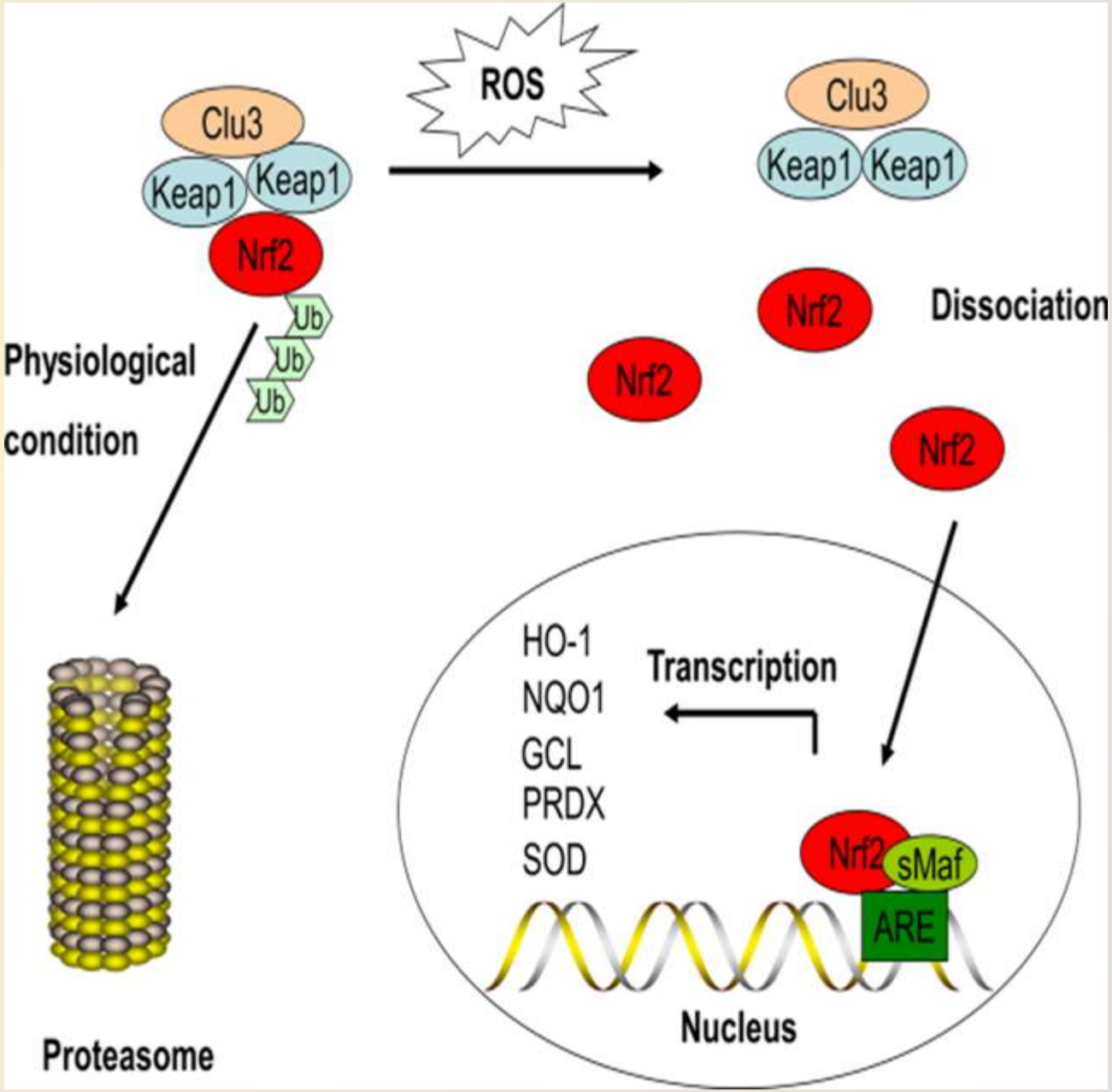

Glyoxalase System
Diabetes
Glucose
$C_6H_{12}O_6$
Glycolysis
α-dicarbonyls
methylglyoxal
GSH
Hemithioacetal
GLO1
S-D-lactoylglutathione
GLO2
D-Lactic Acid
Glycation
AGEs
Proteolytic Pathways (AGEs clearance)
UPS
AUTOPHAGY
inflammation
apoptosis
pericyte dropout
Vascular permeability
BRB breakdown
Healthy Eye
Diabetic Retinopathy

NFE2L2

- The nuclear factor erythroid-2-related factor 2 (NFE2L2) is a transcription factor that is upregulated in times of oxidative stress; and it puts in place a sequence of events that ultimately protect the cell from oxidative injury.
- NFE2L2 activates transcription of antioxidant enzymes by binding to the antioxidant response element (ARE) in the promoter regions of its target genes, in the absence of oxidative stress, the Kelch-like ECH-associated protein 1 (Keap1) can keep NFE2L2 sequestered in the cytosol, where it mediates proteasomal degradation of NFE2L2

NFE2L2

- Upon exposure to ROS, Keap1 undergoes a conformational change that allows NFE2L2 to translocate to the nucleus, bind to the ARE region, where it binds to the ARE and promotes the transcription of numerous genes that encode anti-oxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), hemeoxygenase-1 (HO-1) .
- Glo1 is transcriptionally-regulated by NFE2L2, and the activators of NFE2L2 can promote mRNA and protein expressions of Glo1, and increase Glo1 activity .



Treatment of diabetic retinopathy

- **Systemic Factors Control**

1-The control of blood glucose systemic levels via intensive insulin therapy that significantly reduces the risk for retinopathy prevalence and progression of the disease

2- In hypertensive patients with diabetes, a decrease in systolic blood pressure of 10mmHg was associated with 35% reduction of the risk of progression of DR.

- **Laser photocoagulation**

Pan-retinal photocoagulation (PRP) is a gold standard technology for treating very serious NPDR and PDR. It increased the oxygenation of the tissue, and improves the hypoxia induced by capillary non-perfusion or ischaemia. It reverses the consequences of hypoxia, i.e. VEGF formation and vasodilatation, new vessel formation and oedema.

- **Pharmacotherapies**

VGEF has been the most important factor that has been investigated extensively in relation to the pathophysiology retinal neovascularization and alteration of the BRB.

Drugs that directly inhibit the VEGF molecule include the anti-VEGF (bevacizumab (Avastin), aflibercept intravitreal (Eylea))

- **Surgical intervention**

Targeting oxidative stress as a treatment for DR

- Polyphenols are renowned for their health properties, including antioxidant and anti-inflammatory such as Green tea, Quercetin and Curcumin
- Lutein, Zeaxanthin, Lipoic acid (LA) is a natural thiol antioxidant. LA elevates or maintains cellular GSH levels by acting as a transcriptional inducer of NFE2L2-Gclc-GSH cascade governing GSH synthesis. Treatment with LA increases nuclear NFE2L2 levels and contributes to the binding of NFE2L2 with the antioxidant response element (ARE) to promote the transcription of antioxidant and detoxification genes.
- Sulforaphane is a phytochemical that induces NFE2L2 activation by modifying cysteine residues of Keap.
- Dimethyl fumarate is a synthetic NFE2L2 activator that alkylates Keap1 cysteine residues.

Conclusion

- Oxidative stress is a possible target for pharmacotherapy since it plays a role in the onset and progression of DR.
- Increased levels of MG play a significant role in the pathogenesis of diabetic retinopathy and that activation of Glo1 is an option for treatment.
- Enzymatic antioxidants like SOD, CAT, and GPX and non-enzymatic antioxidants like vitamins E and C and GSH, are important actors in scavenging ROS.
- A possible therapeutic target, the Keap1-NFE2L2-ARE pathway is a crucial defensive mechanism against oxidative stress and xenobiotic damage. DR may be prevented and treated with the aid of small medicines that target the NFE2L2/Keap1 pathways.


Thank
you