ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Review Article

A REVIEW ON ROLE OF MATRIX METALLOPROTEINASES IN THE DEVELOPMENT OF DIABETIC RETINOPATHY

Aggidi Krishna Mohan, Kanala Somasekhar Reddy*, Mala Uday Kiran, Tavarigiri Raghavendra, Chinta Pavan kalyan, Pujari Bhupal

Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research, Autonomous, Chiyyedu (post), Anantapuramu, Andhrapradesh, India

**Corresponding author: somu.reddyvaru@gmail.com*

ABSTRACT

It is a well known fact that diabetes is the worldwide threat, which may lead to multiorgan dysfunction. Diabetes is a chronic metabolic disorder with the hyperglycemia, if not treated, may lead to cause many complications within the body. Diabetic Retinopathy (DR) is a common cause in the chronic diabetes patients. Matrix metalloproteinases (MMPs) are the proteinases that plays crucial role in the development of DR by degrading the extracellular components. Activated gelatinase MMPs (MMPs-2 and MMPs-9) destroy the mitochondria and degrades Blood Retinal Barrier (BRB) from this rises the capillary cell apoptosis in retina. The BRB, which causes DR, is critically affected by MMP-2 and MMP-9. This study will concentrate on the diabetic retinopathy, with the emphasis on MMPs mediated general pathological role, oxidative stress, mitochondrial dysfunction, inflammation, general mechanism involved in the development of DR, and various types of MMPs and angiogenesis.

Keywords: Diabetic retinopathy, Angiogenesis, Apoptosis, Oxidative stress, Matrix metalloproteinases.

1. INTRODUCTION

1.1. Diabetics pose a global challenge

Diabetes mellitus (DM) is associated with hyperglycemia and is due to abnormal carbohydrate metabolism, which relates to relative or absolute impairment in insulin secretion or utilization in the body [1]. Type-2 diabetes is the most prevalent type, and it happens when the body becomes insulin resistant or stops producing insulin. Type 1 diabetes, also known as juvenile diabetes or insulin-dependent diabetes, is a type of diabetes characterised by an insufficient amount of insulin in the body. Insulin deficiency causes abnormal carbohydrate metabolism and blood glucose levels in both situations. When it comes to diabetes diagnosis, type-I is 0.4 percent lower than type-II (7.6%) among the victims [2].

1.2. Diabetic Retinopathy (DR)

The number of persons with diabetic retinopathy (DR) would climb from 126.6 million in 2010 to 191.0 million by 2030, according to the National Centre for Biotechnology Information (NCBI) Knowledge - National Institutes of Health (NIH). Increased vascular permeability causes retinal blood vesicles to weaken owing to the loss of pericytes, resulting in the breakdown of the blood-retinal barrier (BRB) and the flow of plasma from the bloodstream into the retina, inducing edema and hypoxia [3-5]. Mitochondrial damage occurs as a result of hypoxia, and inflammatory mediators such as proteases, growth factors, cytokines, and chemokines such as monocyte chemo attractant protein-1 (MCP-1), interleukin-6 (IL-6), interleukin-8 (IL-8), intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1, cyclooxygenase-2 (COX-2), stromal cell-derived factor-1α (SDF-1α), and prostaglandin E2 production, tumour necrosis factor (TNF), vascular endothelial growth factor (VEGF), matrix metallo-proteinases - 1,2,9 (MMPs). These factors, which are generated in retinal cells as a result of hyperglycemia, interact with one another and trigger separate pathways to aid neovascularization, apoptosis, and fibrosis, resulting in endothelial dysfunction, vision loss, and blindness [4, 6-9]. MMPs are thought to be involved in illnesses such as retinopathy, nephropathy, and cardiomyopathy [10].

1.3. Matrix Metalloproteinases (MMPS)

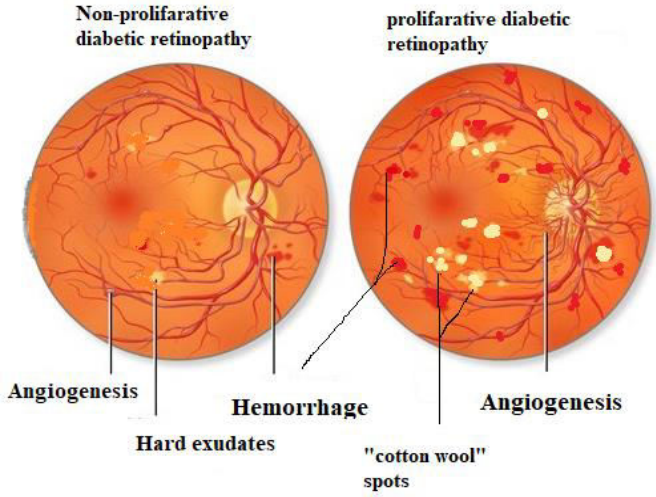
Matrix metalloproteinases (MMPs) are a metalloprotease sub-family including 23 distinct proteases in humans and

24 proteases in mice [11]. Inflammatory disorders, tumour development, and metastasis can all be caused by this set of enzymes, which are responsible for the breakdown of most extracellular matrix proteins during organogenesis. MMPs have three distinct domain structures: MMPs have three related domain structures: a 'pre' region to target for secretion, a 'pro' region to control latency, and an active catalytic region with a zinc-binding active site [12]. Except for polyglycon, MMPs can breakdown all types of ECM. Proteolytic cleavage activates these inactive pro-enzymes that are produced and released as inactive pro-enzymes [11-13]. MMP activity is controlled by endogenous inhibitors such as macroglobulins and tissue inhibitors of metallo-proteinases. TIMP-1 favours MMP-9, while TIMP-2 favours MMP-2, among the TIMP group's four members [14, 15]. MMP-2 promotes endothelial cell migration across the basement membrane and ECM by increasing the front of the migrating column. Membrane type-1 matrix metalloproteinase (MT1-MMP) initiates the activation pathway by converting pro-MMP-2 to an activation intermediate, which then undergoes autocatalytic conversion to create mature MMP-2. MT1-MMP is a cell membrane-bound proteinase with a cytoplasmic tail and a relatively short transmembrane domain, unlike MMPs [16]. It makes easier for these enzymes to bind to certain plasma membrane and intracellular compartment locations. MMP-2 and TIMP-2 join forces to create a tri-molecular complex with MT1-MMP [17-19].

The presence of a comparable cis-regulatory element in the promoters of MMP genes confirms that their cell-specific expression is efficiently regulated. The MMP-9 promoter has numerous functional cis-regulatory areas, which is typical of a gene controlled by many transcription factors. It has AP-1, NF-B, and SP-1 binding sites, and the binding of each, as well as AP-1, controls its expression. Alternative cofactors may be needed for NF-B and AP-1 activation, and poly (ADP-ribose) polymerase-1 (PARP-1), a nuclear chromatin-associated protein, is one of the coactivators for both NF-kB and AP-1 [20, 21]. In addition to their cis-regulatory effects, inflammatory cytokines increase MMP-1 transactivation by enhancing AP-1 binding [22], and multiple triggers, including inflammatory cytokines and growth factors, will coexpress or corepress MMPs. This reaction is often seen several hours after stimulation, implying that the signalling pathway elicited shortly after cellular stimulation acts upstream of the MMP promoters [23]. MMP expression and function are thus tightly regulated at several stages, ranging from zymogen induction to endogenous inhibition and transcriptional activation.

2. SYMPTOMS, STAGES AND ETIOLOGY OF DIABETIC RETINOPATHY

Two main stages of diabetic eye disease are, 1. NPDR (non-proliferative diabetic retinopathy), and 2. PDR (proliferative diabetic retinopathy), symptoms, states and etiology are broadly evolved in the many articles [24-27].



3. BIOLOGICAL ROLE OF MMPS IN DIABETIC RETINOPATHY

3.1. Factors thought to play a role in the development of DR

There is evidence interrelated hyperglycaemia-affected pathways such as inflammation and oxidative stress play a role in the pathobiology of diabetic complications, and several drugs have been developed to treat them. Oxidative stress, polyol pathway activity, advanced glycation end-product (AGE) formation [28], activation of Protein Kinase C (PKC) isoforms, and increased hexosamine pathway flux are among the most studied. Important factors such as lipoprotein-PLA2, pro-inflammatory cytokines (TNF-and IL-1), and secretory phospholipase A2 IIA have been identified in the pathogenesis of DR [29-32].

3.2. Oxidative stress associated with MMPS

An excess of reactive oxidative species (ROS) created as a result of inflammation causes oxidative stress. During the inflammatory process, COX-2, PGE2 and metallo-proteinases (MMPs) are all key mediators [33, 34]. Oxidative stress is caused by an imbalance of free radicals and antioxidants in the body, which can lead to cell and

tissue damage. There is a growing amount of scientific data associating oxidative stress to chronic illnesses such as cancer, diabetes, heart disease, and a variety of others [35].

By functioning as a causative connection between high glucose and metabolic abnormalities, reactive oxygen species are hypothesised to play a role in the development of diabetes complications [36].

Table 1: Main reactive oxygen species:

Name	Formula	Formation
Superoxide	($^{\bullet}O^{-}_{2}$)	Intermediate in 02 reductions to H2O
Singlet oxygen	$^{1}[O_2]$	conversion of triplet energy to molecular oxygen
Hydroxyl radical	(OH^{-})	Powerful oxidant in biological systems
Peroxyl	ROO	↓oxidant ability,↑ diffusibility
Alkoxyl	RO	Ability to oxidise lipids at a moderate rate
Hydrogen peroxide	H_2O_2	Originated from 02
Hypochlorous acid	HClO	Formed through myeloperoxidase action
Single oxygen	lO2	Molecularly excited oxygen through sunlight and radiation
Alpha-oxygen	**α**-O	Formed form oxygen-atom abstraction from N_2O by **α**-Fe catalysts

On the basis of the report by Olguín and Guzman [37-40].

Table 2: Biological effects and specificity of the metalloproteinase

Type of MMPs	Production	Key functions	Other names	References
MMP-1	Endothelial cells, fibroblasts, monocytes	Metastasis, Tissue reshaping	Fibroblast	[38, 39]
MMP-2	myocytes, endothelium and fibroblasts.	Cellular migration, vascularisation, invasion of malignancy and metastases, growth of bones	Gelatinase A	[38, 39]
MMP-3	Epithelial cells, fibroblasts.	Bounding plaque growth, beginning of neoplasm	Stromelysin-1	[38, 39]
MMP-7	Skin glandular epithelial cells, prostate, mammary gland, endometrium	Endometrium involution, innate immunity, wound healing, tumour development, and invasiveness are all factors that influence vascular constriction and cell proliferation.	Matrilysin 1	[38, 39]
MMP-9	Neutrophils, Monocytes	Angiogenesis, cell migration, bone formation, and pro-inflammatory factors all play a role in angiogenesis.	Gelatinase B	[38, 39]
MMP-10	Fibroblasts and epithelial cells	vascular remodelling of atherosclerotic, inflammatory bowel illness	Stromelysin-2	[39]
MMP-12	Osteoclasts, Macrophages	Migration of the macrophage, emphysema	-	[38, 39]
MMP-13	Macrophages	Restoration of the bone	Interstitial collagenase	[38, 39]
MMP-14	Several types of cells	Angiogenesis, cancer transformation, and cell migration are all examples of processes that occur during the development of a tumour.	MT1-MMP	[38, 39]

3.3. MMPS mediated inflammation

In BV2 cells, high hyperglycemia promoted MMP-9 activation, recent studies show that in retina in individuals and animals with diabetic retinopathy MMP-9 and MMP-2 have considerably increased [40, 41]. The inflammatory response generated by diabetes is

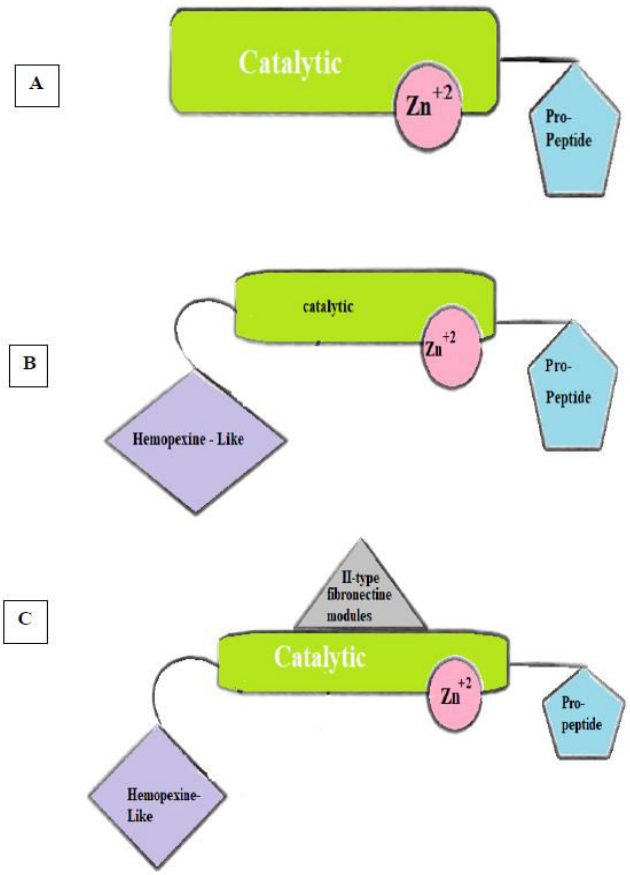
triggered by MMP-9 and the TLR4 signalling pathway in activated microglia [42, 43]. MMPs, which are proactive molecules that increase inflammation and are detected with increased diabetic levels of MCPs, distinguish several members of the chemokine Monocyte Chemoattractant Protein (MCP) family [44, 45].

MMPs have found to orchestrate leukocyte recruitment [6]; In the pathophysiology of diabetic retinopathy, retinal capillaries become ischemic, and the number of leukocytes in the vasculature rises, and these key components of inflammatory processes may be detected in the retina before the histopathology of diabetic retinopathy is investigated in animal models [5]. However, The MMP9 activation mechanism in DR's retina remains unclear [46]. Due to a lack of antioxidant mechanisms in the DR, oxidative damage to the retina and capillary cells is enhanced. Superoxide levels rise when retinal mitochondria become defective and begin to release cytochrome c into the cytoplasm [47]. Over expression of the enzyme that scavenges mitochondrial superoxide (MnSOD) suppresses these diabetes-induced mitochondrial changes and histopathology associated with diabetic retinopathy, indicating that mitochondrial superoxide plays a substantial role in the evolution of diabetic retinopathy [34] MMP-2 and membrane type 1 (MT1)-MMP are vulnerable to oxidative stress; low levels of ROS begin pro-MMPs via oxidation of the sulphide bond in the MMP's prodomain and diminish TIMPs, while peroxynitrite (produced from ROS and nitric oxide) activates pro MMPs by interaction with cytosolic glutathione [48-51]. MMP-2 and MMP-9 levels have been found to be higher in diabetic people and animal models of diabetic retinopathy, and these elevations are thought to contribute to the disordering of all tight-junction complexes and vascular permeability, as well as the maintenance of the blood-retinal barrier [52-55].

4. MMPS: ROLE OF ENZYMES IN ECM DEGRADATION

MMPs are the primary group of enzymes essential for collagen and other extracellular matrix protein (ECM) deterioration (MMPs). Collagen is the major element of the connective tissue and is a major mechanism of degradation for growth, morphogenesis, tissue remodelling, and repair [56-57]. MMPs are zinc dependent endopeptidases which shows enzymatic activity in ECM, except polyglycan all ECM were deteriorate by MMPs [58]. MMPs have similar structure which consist by the 3 domines: s: pro-peptide, proteolytic and hemopexin-like one [59, 60]. Most of MMPs are secreted as inactive pro-enzymes then, the secretion of most MMPs as inactive pro-enzymes, is then followed by the cleavage by plasmin to active forms [61].

Structure of MMPs:



(A) Smallest domain MMP: MMP-7 (B) MMP-1, 3, 8, 10, 12, 13 is a single hemopexin -domain MMPs. (C) MMP-2, 9 gelatinases

Fig. 1: Matrix metalloproteinases' structure

5. MMPS ROLE IN MITOCHONDRIAL DYS-FUNCTION

MMPs cause mitochondrial dysfunction and apoptosis, but the mechanism that disrupts mitochondrial function and leads to the onset of DR is still being researched. As a result of NADPH oxidase activation, during a brief period of hyperglycemia, cytosolic ROS levels rise [62, 63] and with a prolonged rise in ROS, mitochondrial membranes are weakened and mitochondria turn into defective cytochrome C leaks in the cytosol [17-19]. The apoptotic pathway is activated, and retinal capillary cells die, which is a phenomena seen before the histopathology of normal diabetic retinopathy progresses [18, 64-67].

6. MMPS MEDIATED MECHANISM OF ACTIONS

Despite a large number of hypothesised processes derived from many studies, none have been proven in human models. The four major processes in DR include increased polyol pathway fluxes, AGE formation, and PKC and polyol pathway activation [68, 69].

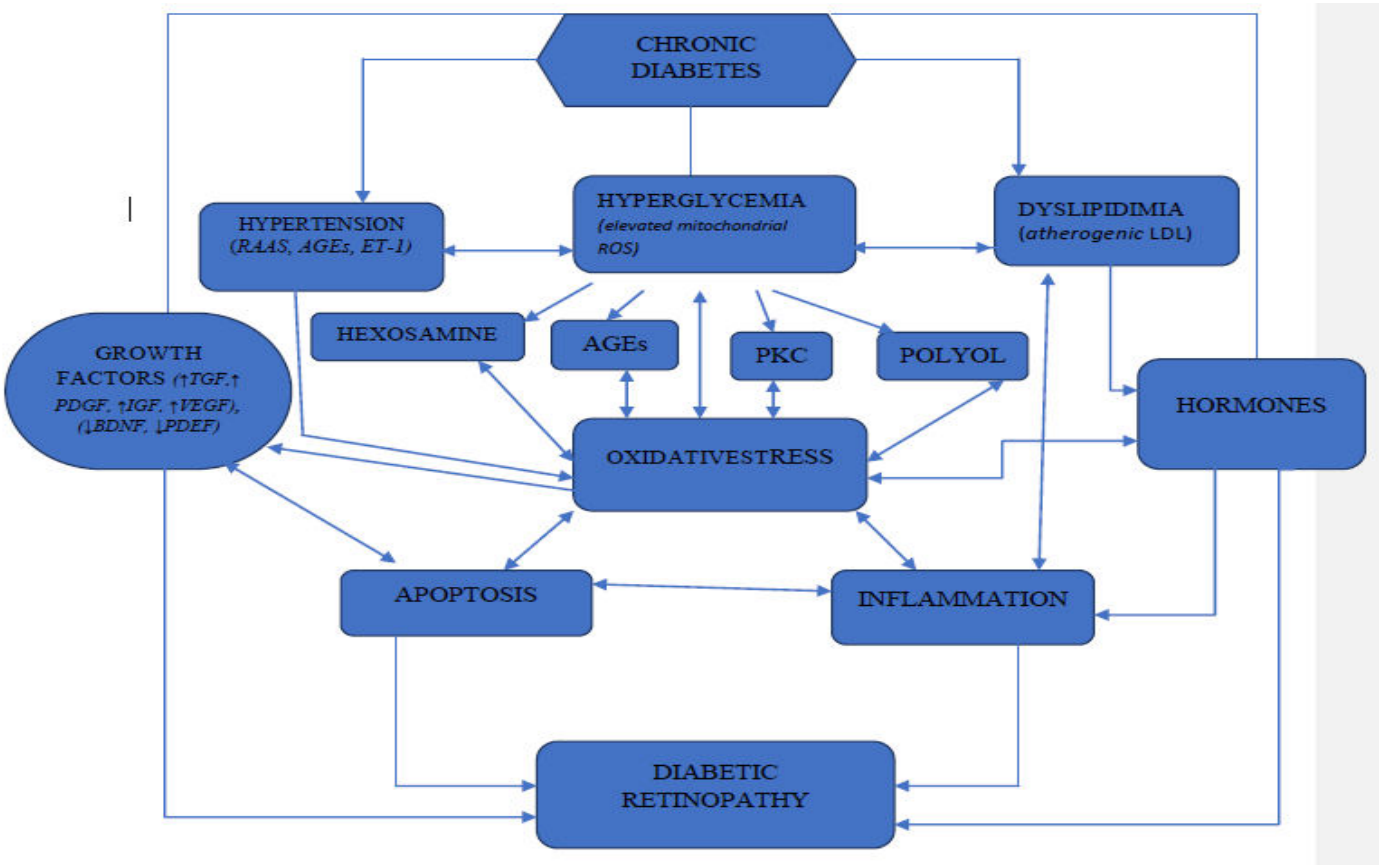


Fig. 2: MMPs Mediated Mechanism of action

7. METALLOPROTEINASE ARE THE FUTURE TARGETS IN THE TREATMENT OF DIABETIC RETINOPATHY

Anti-diabetic medicines and tissue metalloproteinase inhibitors currently available have poor effectiveness in targeting MMP-2 and MMP-9. Basic research to determine the optimum mix of MMP inhibitors is still needed to understand the role of MMPs in DR.

8. ACKNOWLEDGEMENT

The authors express sincere thanks to the management of Raghavendra Institute of Pharmaceutical Education and Research (RIPER).

9. REFERENCES

1. Somasekhar Reddy K, Sudheer A, Pradeepkumar B, Suryaprakash Reddy C. *Indian J Pharmacol.,* 2019; 51:330-336.
2. Roy MS, Klein R, O'Colmain BJ, Klein BE, Moss SE, Kempen JHJAoo. *Arch Ophthalmol.,* 2004; 122**(4):**546-551.
3. Antonetti DA, VanGuilder HD, Mao-Lin C. Vascular permeability in diabetic retinopathy. Diabetic Retinopathy: Springer; 2008; 333-352.
4. Mohammad G, Vandooren J, Siddiquei MM, Martens E, El-Asrar AMA. *Prog Retin Eye Res.,* 2014; 43:76-91.
5. Mohammad G, Siddiquei MM. *J Ocul Biol Dis Infor.,* 2012; 5**(1):**1-8.
6. El-Asrar AMA, Struyf S, Kangave D, Geboes K, Van Damme J. *Eur. Cytokine Netw.,* 2006; 17**(3):**155-165.
7. Descamps FJ, Martens E, Kangave D, Struyf S, Geboes K, Van Damme J, et al. *Exp. Eye Res.,* 2006; 83**(2):**401-407.

8. El-Asrar AMA, Van den Steen PE, Al-Amro SA, Missotten L, Opdenakker G, Geboes K. *Int. Ophthalmol.,* 2007; 27**(1):**11-22.
9. El-Asrar AMA, Nawaz MI, Kangave D, Geboes K, Ola MS, Ahmad S, et al. *Mol.Vis.,* 2011; 17:1829.
10. El-Asrar AMA, Missotten L, Geboes K. *Br J Ophthalmol.,* 2007; 91**(6):**822-826.
11. Klein T, Bischoff R. *Amino acids*, 2011; 41**(2):**271-290.
12. Sorsa T, Tjäderhane L, Konttinen YT, Lauhio A, Salo T, Lee HM, et al. *Annals of medicine*, 2006; 38**(5):**306-321.
13. Fridman R, Toth M, Chvyrkova I, Meroueh SO, Mobashery S. *Cancer and Metastasis Reviews*, 2003; 22**(2):**153-166.
14. Brew K, Dinakarpandian D, Nagase H. *Biochimica et Biophysica Acta*., 2000; 1477**(1-2):**267-283.
15. Kessenbrock K, Plaks V, Werb Z. *Cell*, 2010; 141**(1):**52-67.
16. Stetler-Stevenson WG. *J Clin Invest.,* 1999; 103**(9):**1237-1241.
17. Kowluru RA, Mohammad G, dos Santos JM, Zhong Q. *Diabetes,* 2011; 60**(11):**3023-3033.
18. Kowluru RA, Abbas SN. *Investigative ophthalmology & visual science,* 2003; 44**(12):**5327-5334.
19. Santos JM, Tewari S, Kowluru RA. *Free Radic Biol Med.*, 2012; 53**(9):**1729-1737.
20. Aguilar-Quesada R, Munoz-Gamez J, Martin-Oliva D, Peralta-Leal A, Quiles-Perez R, Rodriguez-Vargas J, et al. *Curr Med Chem.,* 2007; 14**(11):**1179-1187.
21. Zerfaoui M, Errami Y, Naura AS, Suzuki Y, Kim H, Ju J, et al. *J Immun.,* 2010; 185**(3):**1894-1902.
22. Wang X, Bi Z, Chu W, Wan Y. *Int. J. Mol. Med.,* 2005; 16**(6):**1117-1124.
23. Fanjul-Fernández M, Folgueras AR, Cabrera S, López-Otín C. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research,* 2010; 1803**(1):**3-19.
24. Øien GE, Osnes P. *Proc Norwegian Signal Processing Sym,* 1995
25. Candrilli SD, Davis KL, Kan HJ, Lucero MA, Rousculp MD. *J Diabetes Complications,* 2007; 21**(5):**306-314.
26. Yun WL, Acharya UR, Venkatesh YV, Chee C, Min LC, Ng EYK. *Information sciences*, 2008; 178**(1):**106-121.
27. Al-Jarrah MA, Shatnawi H. *J Med Eng Technol.,* 2017; 41(6):498-505.
28. McVicar CM, Ward M, Colhoun LM, Guduric-Fuchs J, Bierhaus A, Fleming T, et al. *Diabetologia,* 2015; 58**(5):**1129-1137.
29. Ola MS, Al-Dosari D, Alhomida AS. *Curr Pharm Des.,* 2018; 24(19):2180-2187.
30. Calderon G, Juarez O, Hernandez G, Punzo S, De la Cruz Z. *Eye*, 2017; 31**(8):**1122-1130.
31. Chakrabarti S, Cukiernik M, Hileeto D, Evans T, Chen S. *Diabetes Metab Res Rev,* 2000; 16**(6):**393-407.
32. Behl T, Kotwani A. *Pharmacol Res,* 2015; 99:137-148.
33. Lu Y, Wahl LM. *J Immun.,* 2005; 175**(8):**5423-5429.
34. Kowluru RA, Kanwar M. *Free Radic Biol Med.,* 2009; 46**(12):**1677-1685.
35. Behl T, Kaur I, Kotwani A. *Survey of ophthalmology,* 2016; 61**(2):**187-196.
36. Brownlee M. *Diabetes*, 2005; 54**(6):**1615-1625.
37. Olguín HJ, Guzmán DC. Free Radicals: Handbook of Free Radicals Formation, Types and Effects: New York, Nova Science Publishers. 2010.
38. Gross J, Lapiere CM. *Proceedings of the National Academy of Sciences of the United States of America,* 1962; 48**(6):**1014.
39. Drankowska J, Kos M, Kościuk A, Marzęda P, Boguszewska-Czubara A, Tylus M, et al.. *Life sciences,* 2019; **229:**149-156.
40. Iannucci J, Rao HV, Grammas P. *Cell Mol Neurobiol.,* 2020:1-12.
41. Mendonca P, Taka E, Soliman KF. *Molecular medicine reports,* 2019; 20**(2):**1736-1746.
42. Zhu S-H, Liu B-Q, Hao M-J, Fan Y-X, Qian C, Teng P, et al. *Inflammation,*2017; 40**(5):**1475-1486.
43. Gorina R, Font-Nieves M, Márquez-Kisinousky L, Santalucia T, Planas AM. *Glia*, 2011; 59**(2):**242-255.
44. Nissinen L, Kähäri V-M. *Biochemica et Biophysica Acta (BBA)-General Subjects,* 2014; 1840**(8):**2571-2580.
45. Kowluru RA, Mishra M. *Prog Mol Biol Transl Sci.,* 2017; 148:67-85.
46. Mishra M, Flaga J, Kowluru RA. *J Cell Physiol.,* 2016; 231**(8):**1709-1718.
47. Djordjevic B, Cvetkovic T, Stoimenov TJ, Despotovic M, Zivanovic S, Basic J, et al. *Eur J Pharmacol.,* 2018; 833:290-297.
48. Pfeilschifter J, Eberhardt W, Huwiler A. *Eur J Pharmacol.,* 2001; **429(1-3)**:279-286.

49. Cox MJ, Sood HS, Hunt MJ, Chandler D, Henegar JR, Aru GM, et al. *Am J Physiol Heart Circ Physiol*., 2002; 282**(4):**H1197-H205.
50. Ho FM, Liu SH, Lin WW, Liau CS. *Journal of cellular biochemistry,* 2007; 101**(2):**442-450.
51. Schulz R. *Annu Rev Pharmacol Toxicol.,* 2007; 47:211-242.
52. Das A, McGuire PG, Eriqat C, Ober RR, DeJuan E, Williams GA, et al. *Invest Ophthalmol Vis Sci*., 1999; 40**(3):**809-813.
53. Jin M, Kashiwagi K, Iizuka Y, Tanaka Y, Imai M, Tsukahara S. *Retina,* 2001; 21**(1):**28-33.
54. Giebel SJ, Menicucci G, McGuire PG, Das A. *Lab Invest,* 2005; 85**(5):**597-607.
55. Navaratna D, McGuire PG, Menicucci G, Das A. *Diabetes,* 2007; 56**(9):**2380-2387.
56. Jabłońska-Trypuć A, Matejczyk M, Rosochacki S. *J Enzyme Inhib Med Chem.,* 2016; 31**(sup1):**177-183.
57. Shapiro SD. *Current opinion in cell biology,* 1998; 10**(5):**602-608.
58. Yang J, Fan X-H, Guan Y-Q, Li Y, Sun W, Yang X-Z, et al. *International journal of ophthalmology,* 2010; 3**(2):**137.
59. Marco M, Fortin C, Fulop T. *J Leukoc Biol.,* 2013; 94**(2):**237-246.
60. Hrabec E, Naduk J, Strek M, Hrabec Z. *Postepy biochemii.,* 2007; 53**(1):**37-45.
61. Barnett JM, McCollum GW, Fowler JA, Duan JJ-W, Kay JD, Liu R-Q, et al. *Invest Ophthalmol Vis Sci.,* 2007; 48**(2):**907-915.
62. Kowluru RA, Kowluru A, Mohammad G, Syed I, Santos JM, et al. *Diabetologia*, 2014; 57**(5):**1047-1056.
63. Kumar B, Kowluru A. *Invest Ophthalmol Vis Sci.,* 2015; 56**(5):**2985-2992.
64. Kowluru RA, Kowluru A, Mishra M, Kumar B. *Progress in retinal and eye research,* 2015; 48:40-61.
65. Mizutani M, Kern TS, Lorenzi M. *J Clin Invest.,* 1996; 97**(12):**2883-2890.
66. Kern TS, Tang J, Mizutani M, Kowluru RA, Nagaraj RH, Romeo G, et al. *Invest Ophthalmol Vis Sci*., 2000; 41**(12):**3972-3978.
67. Kowluru RA. Diabetic retinopathy: mitochondrial dysfunction and retinal capillary cell death. *Antioxid Redox Signal*, 2005; 7**(11-12):**1581.
68. Safi S, Qvist R, Kumar S, Ismail I. *Exp Clin Endocrinol Diabetes,* 2013; 121**(03):**P109.
69. Safi SZ, Qvist R, Kumar S, Batumalaie K, Ismail ISB. *Biomed Res Int*, 2014; 2014.