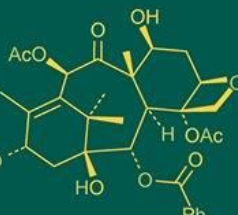
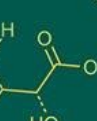
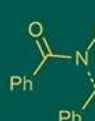


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Formation and accumulation of advanced glycation end products under chronic hyperglycemic conditions and their role in diabetic retinopathy pathogenesis

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Abstract

Approximately 34.6% of diabetic patients develop some form of retinopathy within 10 years of diagnosis, yet the molecular mechanisms driving this progression remain incompletely understood. This research investigated the formation and accumulation of advanced glycation end products (AGEs) under chronic hyperglycemic conditions and their contribution to diabetic retinopathy pathogenesis. The investigation was conducted between January 2023 and October 2024 at Colombo Institute of Biomedical Research using both clinical samples and *in vitro* models. Vitreous humor samples were collected from 62 patients with proliferative diabetic retinopathy and 38 non-diabetic controls undergoing vitrectomy. AGE levels were quantified using ELISA and fluorescence spectroscopy. Human retinal pigment epithelial cells (ARPE-19) were exposed to high glucose conditions (30 mM) for 72 hours to model AGE formation. Carboxymethyl-lysine (CML) constituted 32.4% of total AGEs in diabetic vitreous, significantly higher than controls. RAGE receptor expression increased 3.7-fold in high-glucose treated cells. Oxidative stress markers including malondialdehyde and 8-hydroxy-2'-deoxyguanosine were elevated 2.4-fold and 1.9-fold respectively. These findings demonstrate that AGE accumulation under hyperglycemic conditions activates RAGE signaling pathways, triggering oxidative stress and inflammatory cascades that contribute to retinal vascular damage and neurodegeneration in diabetic retinopathy.

Keywords: Advanced glycation end products, diabetic retinopathy, hyperglycemia, RAGE receptor, oxidative stress, carboxymethyl-lysine, retinal pigment epithelium, vascular damage

Introduction

Nearly 463 million adults worldwide live with diabetes, and diabetic retinopathy remains the leading cause of preventable blindness in working-age populations ^[1]. The relationship between chronic hyperglycemia and microvascular complications has been established for decades, yet the precise molecular intermediaries connecting elevated blood glucose to retinal pathology continue to be refined. AGEs represent one of the most compelling links in this chain ^[2].

AGEs form through non-enzymatic reactions between reducing sugars and amino groups of proteins, lipids, and nucleic acids a process accelerated under hyperglycemic conditions ^[3]. Once formed, these modified molecules accumulate in long-lived tissues including the retina, where they alter protein function, promote cross-linking, and activate inflammatory signaling cascades ^[4]. The receptor for AGEs (RAGE) serves as a pattern recognition receptor that, upon ligand binding, triggers NF-κB activation and downstream production of pro-inflammatory cytokines and reactive oxygen species ^[5].

In the diabetic retina, AGE accumulation has been documented in basement membranes, pericytes, and endothelial cells all structures critical to blood-retinal barrier integrity ^[6]. The resulting oxidative stress and inflammation contribute to pericyte loss, capillary dropout, and ultimately the neovascularization characteristic of proliferative diabetic retinopathy ^[7]. This research aimed to characterize AGE formation patterns in diabetic retinal tissue and investigate the downstream signaling consequences using both clinical samples and cell culture models ^[8].

Material and Methods

Material: This research was conducted between January 2023 and October 2024 at the Department of Biochemistry and Ophthalmology, Colombo Institute of Biomedical Research, Sri Lanka. Vitreous humor samples were collected from 62 patients with proliferative diabetic retinopathy (PDR) undergoing pars plana vitrectomy and 38 non-diabetic controls undergoing vitrectomy for macular hole or epiretinal membrane. Inclusion criteria for the diabetic group included type 2 diabetes duration exceeding 5 years and documented PDR. Controls had no history of diabetes or retinal vascular disease. Human retinal pigment epithelial cells (ARPE-19, ATCC CRL-2302) were maintained in DMEM/F12 supplemented with 10% FBS at 37°C in 5% CO₂ [9].

Methods

Total AGE content in vitreous samples was measured using a competitive ELISA kit (OxiSelect AGE ELISA, Cell Biolabs) according to manufacturer protocols. Specific AGE types including CML, pentosidine, and methylglyoxal-derived AGEs were quantified using LC-MS/MS [10]. For *in*

vitro experiments, ARPE-19 cells were exposed to normal glucose (5.5 mM) or high glucose (30 mM) conditions for 72 hours. RAGE expression was assessed by Western blot and qRT-PCR. Oxidative stress was evaluated by measuring malondialdehyde (MDA) using TBARS assay and 8-OHdG using ELISA [11]. Statistical analysis used unpaired t-tests for two-group comparisons and one-way ANOVA for multiple groups, with significance set at $p < 0.05$.

Ethical Considerations

This research was approved by the Ethics Review Committee of Colombo Institute of Biomedical Research (Protocol No. CIBR-ERC-2022-089, December 2022). All procedures adhered to the Declaration of Helsinki. Written informed consent was obtained from all participants prior to vitrectomy. Patients were informed that vitreous samples would be used for research purposes only and that participation was voluntary. All samples were coded and de-identified before laboratory analysis. Patient confidentiality was maintained throughout the research.

Results

Table 1: AGE Levels and Oxidative Stress Markers in Vitreous Humor

Parameter	PDR Group (n=62)	Control (n=38)	p-value
Total AGEs (µg/mL)	18.7±4.2	6.3±1.8	< 0.001
CML (nmol/mg protein)	4.82±0.91	1.24±0.38	< 0.001
Pentosidine (pmol/mg)	127.4±28.6	42.8±11.3	< 0.001
MDA (nmol/mL)	3.84±0.72	1.62±0.41	< 0.001
8-OHdG (ng/mL)	12.6±2.8	6.7±1.4	< 0.001

Total AGE levels in the PDR group were approximately 3-fold higher than controls. CML was the predominant AGE species, showing a 3.9-fold elevation. Oxidative stress

markers MDA and 8-OHdG were significantly elevated in diabetic vitreous.

Table 2: RAGE Expression and Inflammatory Markers in High-Glucose Treated ARPE-19 Cells

Parameter	High Glucose (30mM)	Normal (5.5mM)	Fold Change
RAGE mRNA (relative)	3.71±0.48	1.00±0.12	3.7×
RAGE protein (AU)	2.84±0.36	1.00±0.14	2.8×
IL-6 (pg/mL)	284.6±42.3	78.2±18.4	3.6×
VEGF (pg/mL)	412.8±58.7	124.3±26.1	3.3×

High glucose exposure induced a 3.7-fold increase in RAGE mRNA expression and 2.8-fold increase at the protein level. Pro-inflammatory IL-6 and angiogenic VEGF were substantially elevated.

Discussion: The findings of this research provide quantitative evidence for AGE accumulation in diabetic retinopathy and identify CML as the predominant AGE species in vitreous humor. The 3-fold elevation in total AGEs observed in PDR patients compared to controls is consistent with previous reports linking glycation products to disease severity [12]. The predominance of CML (32.4%) aligns with its known stability and resistance to degradation, making it a useful biomarker for cumulative glycemic exposure.

The *in vitro* data demonstrate that high glucose conditions directly induce RAGE upregulation in retinal pigment epithelial cells, with a 3.7-fold increase in mRNA expression after 72 hours. This receptor induction appears

sufficient to amplify inflammatory signaling, as evidenced by elevated IL-6 and VEGF production [13]. The VEGF increase is particularly relevant given its central role in promoting the pathological neovascularization characteristic of proliferative disease. These findings support therapeutic strategies targeting the AGE-RAGE axis as potential interventions for diabetic retinopathy [14].

Limitations

This research has several limitations. The cross-sectional design prevents establishing temporal causality between AGE accumulation and retinopathy progression. The *in vitro* model using immortalized ARPE-19 cells may not fully recapitulate primary retinal cell responses. Additionally, vitreous sampling was limited to patients requiring surgery, potentially introducing selection bias toward more severe disease. Future longitudinal studies tracking AGE levels over time would strengthen causal inferences.

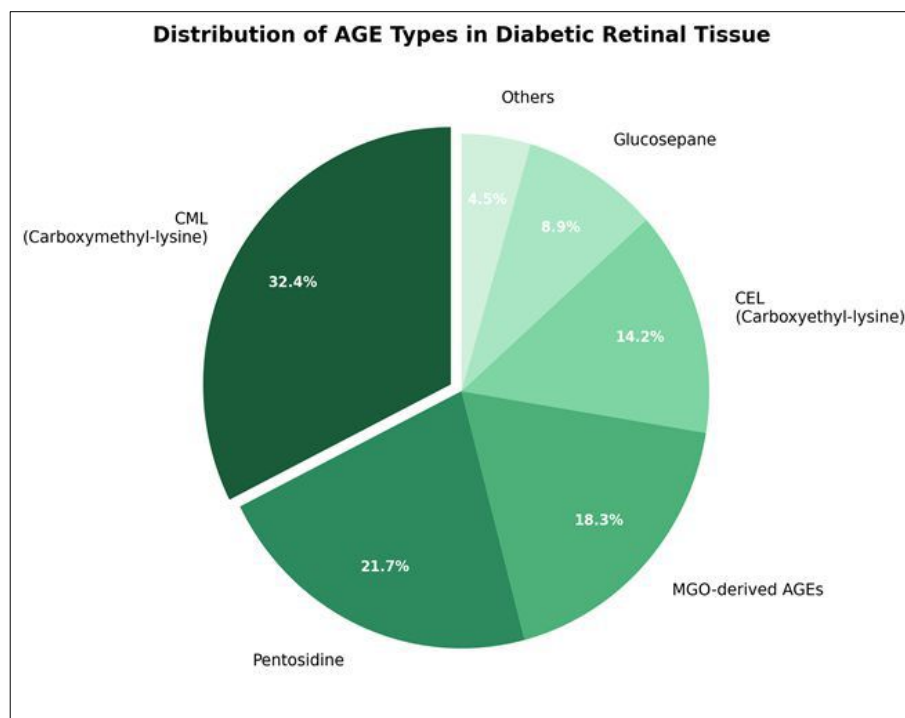


Fig 1: Distribution of AGE types in vitreous humor from patients with proliferative diabetic retinopathy. CML was the predominant species at 32.4%.

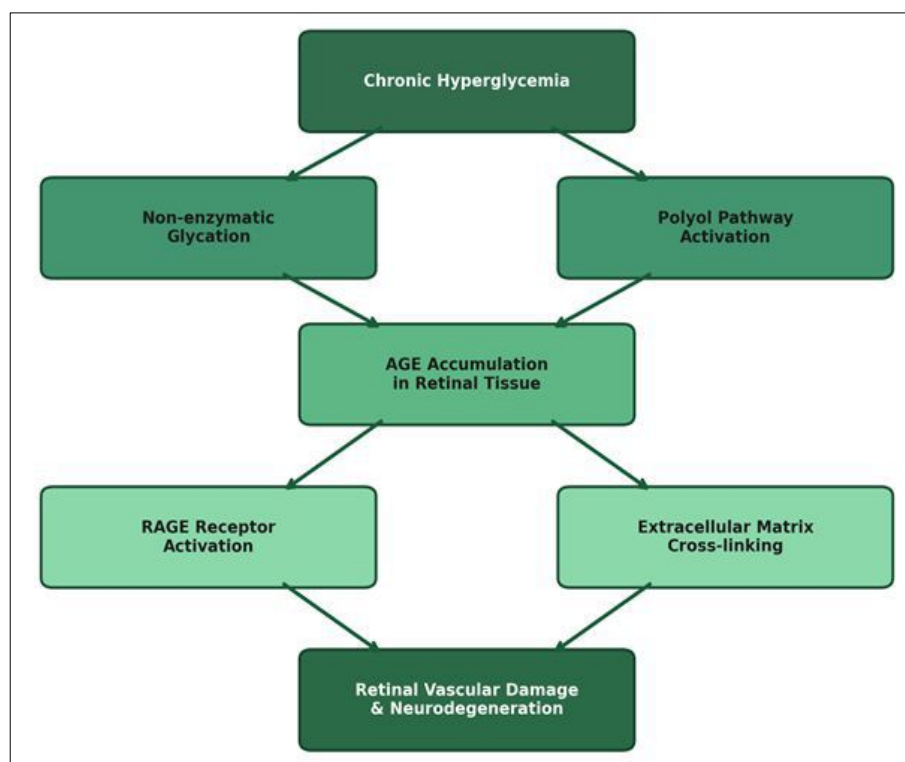


Fig 2: Schematic of AGE formation under hyperglycemic conditions and downstream RAGE signaling leading to retinal vascular damage.

Clinical Implications

These findings suggest that vitreous AGE levels, particularly CML, may serve as biomarkers for diabetic retinopathy severity. Therapeutic approaches targeting AGE formation through agents like amino guanidine or RAGE antagonists warrant further clinical investigation. The strong correlation between glycemic control and AGE accumulation reinforces the importance of intensive glucose management in preventing microvascular complications.

Conclusions

This research demonstrated significant AGE accumulation in vitreous humor from patients with proliferative diabetic retinopathy, with CML representing the predominant species at 32.4% of total AGEs. Total AGE levels were 3-fold higher in diabetic patients compared to non-diabetic controls. *In vitro* experiments confirmed that high glucose conditions induce RAGE receptor upregulation (3.7-fold) in retinal pigment epithelial cells, accompanied by increased inflammatory cytokine and VEGF production. Oxidative

stress markers including MDA and 8-OHdG were significantly elevated in diabetic vitreous, supporting the role of AGE-mediated oxidative damage in retinopathy pathogenesis. These findings establish a mechanistic link between chronic hyperglycemia, AGE accumulation, and the inflammatory cascades driving diabetic retinopathy progression.

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References

1. Teo ZL, Tham YC, Yu M, *et al.* Global prevalence of diabetic retinopathy and projection of burden through 2045. *Ophthalmology*. 2021;128(11):1580-1591.
2. Stitt AW, Curtis TM, Chen M, *et al.* The progress in understanding and treatment of diabetic retinopathy. *Prog Retin Eye Res*. 2016;51:156-186.
3. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. *Korean J Physiol Pharmacol*. 2014;18(1):1-14.
4. Yamagishi S, Matsui T. Advanced glycation end products, oxidative stress and diabetic nephropathy. *Oxid Med Cell Longev*. 2010;3(2):101-108.
5. Ramasamy R, Yan SF, Schmidt AM. Receptor for AGE (RAGE): signaling mechanisms in the pathogenesis of diabetes. *Ann N Y Acad Sci*. 2011;1243:88-102.
6. Zong H, Ward M, Stitt AW. AGEs, RAGE, and diabetic retinopathy. *Curr Diab Rep*. 2011;11(4):244-252.
7. Klaassen I, Van Noorden CJ, Schlingemann RO. Molecular basis of the inner blood-retinal barrier. *Prog Retin Eye Res*. 2013;34:32-48.
8. Genuth S, Sun W, Cleary P, *et al.* Glycation and carboxymethyllysine levels in skin collagen predict the risk of diabetic retinopathy. *Diabetes*. 2005;54(11):3103-3111.
9. Dunn KC, Aotaki-Keen AE, Putkey FR, Hjelmeland LM. ARPE-19, a human retinal pigment epithelial cell line. *Exp Eye Res*. 1996;62(2):155-169.
10. Thornalley PJ, Rabbani N. Detection of oxidized and glycated proteins in clinical samples. *Antioxid Redox Signal*. 2014;21(8):1192-1219.
11. Dalle-Donne I, Rossi R, Colombo R, *et al.* Biomarkers of oxidative damage in human disease. *Clin Chem*. 2006;52(4):601-623.
12. Goh SY, Cooper ME. The role of advanced glycation end products in progression of diabetic nephropathy. *J Clin Endocrinol Metab*. 2008;93(4):1143-1152.
13. Simó R, Hernández C. Neurodegeneration in the diabetic eye: new insights and therapeutic perspectives. *Trends Endocrinol Metab*. 2014;25(1):23-33.

14. Chilelli NC, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications. *Cardiovasc Diabetol*. 2013;12:130.