

# Elevated advanced glycation end products are associated with subfoveal ellipsoid zone disruption in diabetic macular edema

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**Purpose:** Advanced glycation end products (AGEs), due to increased production and a slow turnover rate, serve as mediators of “metabolic memory” even after the resolution of hyperglycemia. A prospective study was undertaken to evaluate the association of AGEs with subfoveal ellipsoid zone (EZ) disruption in diabetic macular edema (DME). **Methods:** A tertiary-care-center-based cross-sectional study included 40 consecutive cases with DME and 20 healthy controls in the age group of 40–65 years. All the study subjects underwent spectral-domain optical coherence tomography (SD-OCT) for cross-sectional imaging of the retina. The EZ was defined as a hyperreflective band below the external limiting membrane. The disruption of EZ was graded as intact EZ and disrupted EZ. Serum AGEs were assessed by assay of N<sup>ε</sup>-carboxymethyl-lysine (N<sup>ε</sup>-CML) using the standard protocol. Data were analyzed statistically. **Results:** Subfoveal EZ disruption was noted in 80% (32/40) of the cases of DME. In the cases without EZ disruption, visual acuity (LogMAR VA) was 0.60 ± 0.52, whereas in cases with EZ disruption, LogMAR VA was 0.96 ± 0.56 ( $P < 0.001$ ). In the cases without EZ disruption, N<sup>ε</sup>-CML was 94.31 ± 57 ng/mL, whereas in cases with EZ disruption N<sup>ε</sup>-CML was 120.64 ± 71.98 ng/mL ( $P < 0.001$ ). **Conclusion:** In DME, increased levels of AGEs are significantly associated with EZ disruption on SD-OCT.

**Key words:** Advanced glycation end products, diabetic macular edema, diabetic retinopathy, EZ, spectral-domain optical coherence tomography

Diabetes mellitus (DM) is a leading cause of morbidity and mortality in both developing and developed nations. Diabetic retinopathy (DR), microangiopathy is an important cause of preventable blindness affecting 93 million people worldwide of which 21 million have a treatable form of diabetic macula edema (DME).<sup>[1]</sup> The pathogenesis of DR is a complex process in which hyperglycemia plays a major role.<sup>[2]</sup>

Hyperglycemia results in deranged carbohydrate metabolism which results in the formation of advanced glycation end products (AGEs). AGEs are formed by a non-enzymatic reaction between reducing sugars and amine residues on proteins. AGEs also originate from exogenous dietary sources. A ‘low glycemic index-low AGE’ therapeutic diet has been proposed for reducing the severity of DR.<sup>[3]</sup> AGEs contribute to the pathogenesis of DR at the metabolic, vascular, and inflammatory levels.<sup>[4-6]</sup> N<sup>ε</sup>-carboxymethyl-lysine (N<sup>ε</sup>-CML) is one of the most prevalent AGEs and has been established to be associated with the severity of DR.<sup>[7,8]</sup>

The cross-sectional imaging of the retina obtained from spectral-domain optical coherence tomography (SD-OCT) provides reliable, non-invasive *in vivo* retinal histology. The superior delineation of the fine structures on SD-OCT images has enabled the evaluation of photoreceptor ellipsoid zone (EZ)

in detail which is an important prognostic factor of visual outcome in DR.<sup>[9]</sup>

The present study was undertaken to evaluate the association of AGEs with subfoveal disruption of EZ in DME.

## Methods

The authors confirm adherence to the tenets of the Declaration of Helsinki. The study was undertaken after the institutional review board clearance and a written informed voluntary consent from all the study subjects. The study was a tertiary-care-center-based cross-sectional study of the cases of type 2 DM with DME and healthy controls. The consecutive cases of type 2 DM in the age group of 40–65 years were included. After sample size calculation, based on the Early Treatment of Diabetic Retinopathy Study (ETDRS) classification, 40 cases with DME (non-proliferative DR,  $n = 20$  and proliferative DR,  $n = 20$ ) and 20 healthy controls were included. DME was classified based on the clinical criteria of the ETDRS classification.<sup>[10,11]</sup> The patients with any other ocular or systemic diseases affecting the retinal vascular pathology, previous intravitreal injection(s), ophthalmic surgical or laser interventions, vitreous hemorrhage, and tractional retinal

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detachment were not included. The patients having conditions in which serum level of AGEs is raised like the Alzheimer's disease, pulmonary fibrosis, atherosclerosis, end-stage renal disease, and tobacco smoking were also excluded.

Information regarding the patient's age, gender, and disease duration was also recorded. Glycated hemoglobin (HbA1c) was estimated for long-term glycemic control. All the study subjects underwent detailed ophthalmological examination including visual acuity (VA) assessment using the LogMAR scale (LogMAR VA). The fundus evaluation was done using the stereoscopic slit-lamp biomicroscope using a 90 D lens and indirect ophthalmoscopy. The digital fundus photography and fluorescein angiography were performed in all the cases using the Zeiss fundus camera FF 450 Plus with a pixel width of 0.0054 and image size 2588 × 195 (Carl Zeiss Meditec, Jena, Germany). Every study subject underwent SD-OCT (Cirrus high-definition OCT, Carl Zeiss Meditec Inc., CA, USA). The macular thickness analysis was done using the macular cube 512 × 128 feature. *In vivo* macular edema was also defined based on the Cochrane database, using the macular central subfield thickness (CST).<sup>[12]</sup> On cross-sectional imaging, EZ was defined as a hyperreflective band below the external limiting membrane. The disruption of EZ was graded as no disruption of EZ and disrupted EZ in the subfoveal area [Fig. 1]. Two experienced observers masked to the status of DR were involved in the SD-OCT assessment.

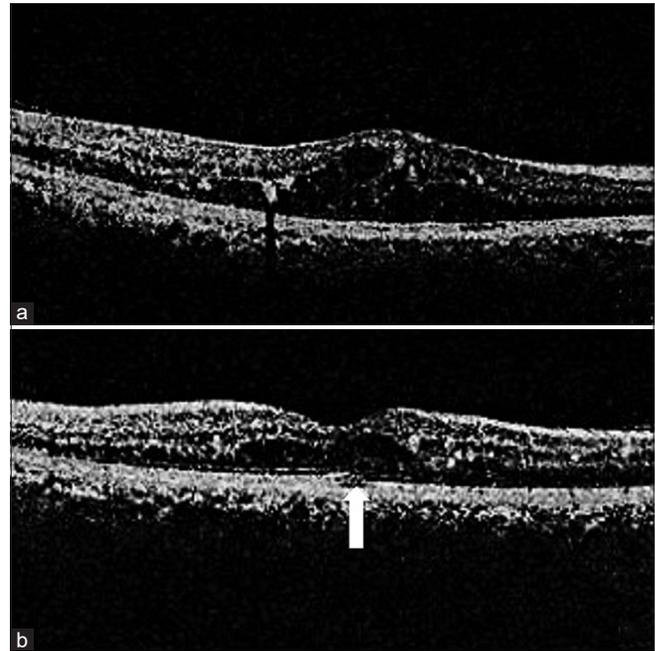
AGEs were assessed using assay N<sup>e</sup>-CML. The blood samples of 7 mL were collected from the study subjects. HbA1c was measured on an autoanalyzer using the standard protocol. Subsequently, 50 µL serum was used for the assay of N<sup>e</sup>-CML using the standard protocol. Human N<sup>e</sup>-CML Enzyme Linked Immunosorbent Assay (ELISA) kit procured from the USCN, Life Science Inc., Houston, USA and ELISA plate reader (Synergy HT, Biotech, USA) were utilized. The concentration of N<sup>e</sup>-CML in the serum sample was calculated based on the standard curve. The values were expressed as ng/mL.

### Statistics

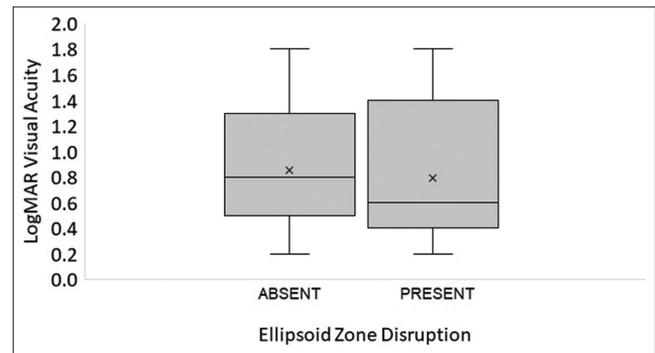
Two sample t-tests and a Chi-square test were used for the statistical analysis. The interobserver correlation was also calculated using Spearman's correlation analysis with  $P < 0.05$  being considered statistically significant.

## Results

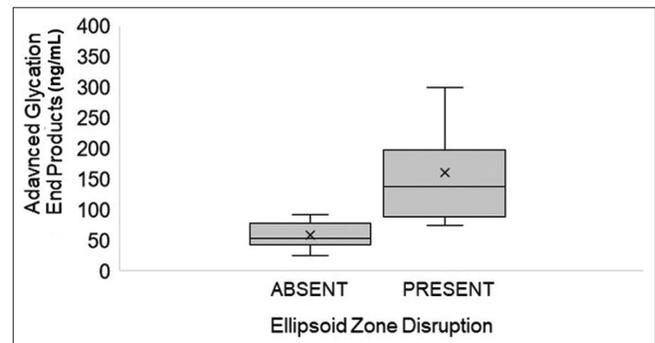
The demographic and clinical data are summarized in Table 1. The values are expressed as mean ± standard deviation. No statistical difference was observed in the age and gender distribution between the cases and controls making both the groups comparable ( $P > 0.05$ ). The interobserver agreement was 0.90. The EZ disruption was not observed in the controls. Out of the 40 cases, EZ disruption was noted in 32 cases (Non-Proliferative Diabetic Retinopathy [NPDR],  $n = 15$ ; PDR,  $n = 17$ ). LogMAR VA in the controls was  $0.13 \pm 0.22$ . In the cases without EZ disruption, the LogMAR VA was  $0.60 \pm 0.52$ , whereas in the cases with EZ disruption, the LogMAR VA was  $0.96 \pm 0.56$ . Fig. 2 shows the distribution of VA among the cases with and without EZ disruption. A statistically significant difference was observed between the cases with and without EZ disruption ( $P < 0.001$ ).



**Figure 1:** (a) SD-OCT showing DME on the cross-sectional image along with an intact subfoveal EZ. (b) SD-OCT showing DME on the cross-sectional image along with subfoveal EZ disruption (indicated by an arrow)



**Figure 2:** Box and Whisker plot illustrating the distribution of visual acuity among cases with and without ellipsoid zone disruption ('x' showing the mean)



**Figure 3:** Box and Whisker plot illustrating the distribution of serum advanced glycation end products among the cases with and without ellipsoid zone disruption ('x' showing the mean)

CST in the controls was  $241.65 \pm 35.96$ . In cases without EZ disruption, the CST was  $289.38 \pm 29.75$  microns, whereas

**Table 1: Summary of demographic and clinical data. Values are expressed as mean±standard deviation**

Variables	Controls	Cases	
		Intact ellipsoid zone (n=8)	Disrupted ellipsoid zone (n=32)
Age	49.45±0.50	54.25±5.63	50.56±8.42
Gender			
Male	12	6	21
Female	8	2	11
LogMAR VA	0.13±0.22	0.60±0.52	0.96±0.56
HbA1c	6.18±0.62	7.52±1.09	8.31±1.61
CST	241.65±35.96	289.38±29.75	327.84±36.26
N <sup>e</sup> -CML	29.63±18.62	94.31±57.15	120.64±71.98

HbA1c: Glycated hemoglobin; CST: Central subfield thickness, N<sup>e</sup>-CML: N<sup>e</sup>-carboxymethyl-lysine

in the cases with EZ disruption, the CST was 327.84 ± 36.26 microns.

Fig. 3 shows the distribution of N<sup>e</sup>-CML among the cases with and without EZ disruption. N<sup>e</sup>-CML in the controls was 29.63 ± 18.62 ng/mL. In the cases without EZ disruption, N<sup>e</sup>-CML was 94.31 ± 57 ng/mL, whereas in the cases with EZ disruption, N<sup>e</sup>-CML was 120.64 ± 71.98 ng/mL. A statistically significant difference was observed between the cases with and without EZ disruption ( $P < 0.001$ ).

## Discussion

AGEs and their receptors play a major role in the pathogenesis of both the micro as well as macrovascular complications of DM.<sup>[13]</sup> AGEs are considered as promising biomarkers of late diabetic complications.<sup>[14]</sup> Due to the increased production of AGEs and a slow turnover rate, they serve as the mediators of “metabolic memory” even after the hyperglycemia is resolved.<sup>[15]</sup> The activation of AGE receptors induces multiple signaling pathways that increase inflammation, oxidative stress, enhanced calcium deposition, and increased vascular smooth muscle apoptosis. AGE-oxidative stress axis and its cross-talk with the renin-angiotensin system contribute to vascular complications.<sup>[15]</sup>

The EZ serves as a clinical indicator of photoreceptor integrity. Biologically, EZ consists of the mitochondria mainly enabling higher levels of energy consumption within the photoreceptors.<sup>[16]</sup> Mitochondrial dysfunction in the photoreceptors at the fovea results in reduced VA in DME.<sup>[17]</sup> The absence of the subfoveal EZ on SD-OCT corresponds to the reduced reflectivity or anatomic absence of the EZ. In the present study, a statistically significant difference was observed in the serum levels of AGEs between the cases with and without subfoveal EZ disruption.

The effect of the increased levels of AGEs on EZ disruption is multifactorial. AGEs have an impact through their effect on retinal microvasculature, vascular endothelial growth factor (VEGF), intercellular adhesion molecule-1 (ICAM 1), nitrosative and oxidative stress, and vitamin D and calcium metabolism.

The accumulation of AGEs and their receptor activation lead to endothelial dysfunction, the release of inflammatory cytokines, and proangiogenic factors, resulting in pericyte apoptosis, vascular inflammation, and angiogenesis, as well as the breakdown of the inner blood-retinal barrier, thus,

damaging the vascular components of the retina.<sup>[18]</sup> This leads to leakage and fluid accumulation resulting in macular edema. Boehm *et al.*<sup>[19]</sup> found that AGEs cause clinical macular edema. In the present study, an increase in the CST was documented on SD-OCT with increased levels of AGEs.

AGEs increase the expression of VEGF in the retinal pericytes and Retinal Pigment Epithelium (RPE) cells.<sup>[20,21]</sup> Retinal VEGF induces ICAM-1 expression leading to leukostasis and the breakdown of the blood-retinal barrier.<sup>[6]</sup> Jain *et al.*<sup>[22]</sup> demonstrated the correlation of VEGF and ICAM-1 on EZ disruption. AGEs induce oxidative stress generation resulting in inflammatory and thrombogenic reactions. The mitochondrial superoxide generation plays an important role in the formation and accumulation of AGEs under diabetic conditions.<sup>[23]</sup> Sharma *et al.*<sup>[24]</sup> highlighted nitric oxide and oxidative stress in DR to be associated with the disruption of EZ. The increased serum anti-myeloperoxidase antibody levels have been found to be associated with EZ disruption and decreased VA in DR.<sup>[25]</sup>

The increased levels of AGEs have also been correlated with deranged metabolism thereby leading to compromised nutritional status.<sup>[26]</sup> Vitamin D deficiency has been found to be associated with disruption EZ.<sup>[27]</sup> Vitamin D also plays an important role in regulating calcium homeostasis. The increased serum-ionized calcium induces retinal photoreceptor apoptosis resulting in EZ disruption in DR.<sup>[28]</sup>

The disruption of photoreceptor EZ serves as a marker of visual outcome in DR.<sup>[9]</sup> In the present study, a statistically significant difference in LogMAR VA was observed between the cases with and without subfoveal EZ disruption. Our earlier study<sup>[8]</sup> highlighted the association of the increasing levels of serum AGEs and glycosylated hemoglobin with ETDRS grades of DR.

Several novel therapeutic strategies targeting AGEs and receptors for advanced glycation end products (RAGE) are being developed. These therapeutic options include AGE cross-link breakers, AGE inhibitors, and RAGE antagonists.<sup>[29]</sup>

## Conclusion

AGEs are the new-age tools of translational medicine which is defined as “an interdisciplinary branch of the biomedical field supported by three main pillars: bench side, bedside, and community.”<sup>[30]</sup> The estimation of AGEs from the blood

levels serves as a bench side examination, EZ disruption on SD-OCT serves as a bedside diagnostic tool, and the therapeutic agent targeting AGE and RAGE serves the community.

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#### Conflicts of interest

There are no conflicts of interest.

#### References

1. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, *et al.*; Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. *Diabetes Care* 2012;35:556-64.
2. Hanssen KF. Blood glucose control and microvascular and macrovascular complications. *Diabetes* 1997;46:101-3.
3. Vlassara H, Uribarri J. Advanced glycation end products (AGE) and diabetes: Cause, effect, or both? *Curr Diab Rep* 2014;14:453.
4. Frederick P, Yuxi F, Franziska H, Sigrid H, Grietje M, Hillebrands J, *et al.* Pericyte migration: A novel mechanism of pericyte loss in experimental diabetic retinopathy. *Diabetes* 2008;57:2495-502.
5. Puddu A, Sanguineti R, Durante A, Nicolò M, Viviani GL. Vascular endothelial growth factor-C secretion is increased by advanced glycation end-products: Possible implication in ocular neovascularization. *Mol Vis* 2012;18:2509-17.
6. Vlassara H, Cai W, Crandall J, Goldberg T, Oberstein R, Dardaine V, *et al.* Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetic angiopathy. *Proc Natl Acad Sci USA* 2002;99:15596-601.
7. Hirata K, Kubo K. Relationship between blood levels of N-carboxymethyl-lysine and pentosidine and the severity of microangiopathy in type 2 diabetes. *Endocr J* 2004;51:537-44.
8. Mishra N, Saxena S, Shukla RK, Singh V, Meyer CH, Kruzliak P, *et al.* Association of serum N(ε)-Carboxy methyl lysine with severity of diabetic retinopathy. *J Diabetes Complications* 2016;30:511-7.
9. Maheshwary AS, Oster SF, Yuson RMS, Cheng L, Mojana F, Freeman WR. The association between percent disruption of the photoreceptor inner segment-outer segment junction and visual acuity in diabetic macular edema. *Am J Ophthalmol* 2010;150:63-7.
10. Early Treatment Diabetic Retinopathy Study Research Group. Grading diabetic retinopathy from stereoscopic color fundus photographs--An extension of the modified Airlie House classification. ETDRS report number 10. *Ophthalmology* 1991;98:786-806.
11. Gangnon RE, Davis MD, Hubbard LD, Aiello LM, Chew EY, Ferris FL 3<sup>rd</sup>, *et al.*; Early Treatment Diabetic Retinopathy Study Research Group. A severity scale for diabetic macular edema developed from ETDRS data. *Invest Ophthalmol Vis Sci* 2008;49:5041-7.
12. Virgili G, Menchini F, Murro V, Peluso E, Rosa F, Casazza G. Optical coherence tomography (OCT) for detection of macular oedema in patients with diabetic retinopathy. *Cochrane Database Syst Rev* 2011;1:CD008081.
13. Omsland TK, Bangstad HJ, Berg TJ, Kolset SO. Avanserte glykerte endprodukter og hyperglykemi [Advanced glycation end products and hyperglycaemia]. *Tidsskr Nor Laegeforen* 2006;126:155-8.
14. Simó-Servat O, Planas A, Ciudin A, Simó R, Hernández C. Assessment of advanced glycation end-products as a biomarker of diabetic outcomes. *Endocrinol Diabetes Nutr (Engl Ed)* 2018;65:540-5.
15. Fishman SL, Sonmez H, Basman C, Singh V, Poretzky L. The role of advanced glycation end-products in the development of coronary artery disease in patients with and without diabetes mellitus: A review. *Mol Med* 2018;24:59.
16. Mori Y, Suzuma K, Uji A, Ishihara K, Yoshitake S, Fujimoto M, *et al.* Restoration of foveal photoreceptors after intravitreal ranibizumab injections for diabetic macular edema. *Sci Rep* 2016;6:39161. doi: 10.1038/srep39161. PMID: 27966644; PMCID: PMC5155247.
17. Saxena S, Akduman L, Meyer CH. External limiting membrane: Retinal structural barrier in diabetic macular edema. *Int J Retina Vitreous* 2021;7:16.
18. Xu J, Chen LJ, Yu J, Wang HJ, Zhang F, Liu Q, *et al.* Involvement of advanced glycation end products in the pathogenesis of diabetic retinopathy. *Cell Physiol Biochem* 2018;48:705-17.
19. Boehm BO, Schilling S, Rosinger S, Lang GE, Lang GK, Kientsch R. Elevated serum levels of N ε-carboxymethyl-lysine, an advanced glycation end product, are associated with proliferative diabetic retinopathy and macular oedema. *Diabetologia* 2004;47:1376-9.
20. Yamagishi S, Amano S, Inagaki Y. Advanced glycation end products induced apoptosis and overexpression of vascular endothelial growth factor in bovine retinal pericytes. *Biochem Biophys Res Commun* 2002;290:973-8.
21. Lu M, Kuroki M, Amano S, Tolentino M, Keough K, Kim I, *et al.* Advanced glycation end products increase retinal vascular endothelial growth factor expression. *J Clin Invest* 1998;101:1219-24.
22. Jain A, Saxena S, Khanna VK, Shukla RK, Meyer CH. Status of serum VEGF and ICAM-1 and its association with external limiting membrane and inner segment-outer segment junction disruption in type 2 diabetes mellitus. *Mol Vis* 2013;19:1760-8.
23. Yamagishi S, Maeda S, Matsui T, Ueda S, Fukami K, Okuda S. Role of advanced glycation end products (AGEs) and oxidative stress in vascular complications in diabetes. *Biochim Biophys Acta* 2012;1820:663-71.
24. Sharma S, Saxena S, Srivastav K, Shukla RK, Mishra N, Meyer CH, *et al.* Nitric oxide and oxidative stress is associated with severity of diabetic retinopathy and retinal structural alterations. *Clin Exp Ophthalmol* 2015;43:429-36.
25. Sinha S, Saxena S, Prasad S, Mahdi AA, Bhasker SK, Das S, *et al.* Association of serum levels of anti-myeloperoxidase antibody with retinal photoreceptor ellipsoid zone disruption in diabetic retinopathy. *J Diabetes Complications* 2017;31:864-8.
26. Sharma Y, Saxena S, Saxena A, Mishra A, Natu SM. Interrelationship of elevated serum Advanced Glycation End-product levels and malnutrition (Subjective Global Assessment) scores with the severity of retinopathy in type II diabetes. *Clin Nutr ESPEN* 2015;10:e42-8.
27. Nadri G, Saxena S, Kaur A, Ahmad K, Garg P, Mahdi AA, *et al.* Correlation between vitamin D serum levels and severity of diabetic retinopathy in patients with type 2 diabetes mellitus. *J Endocrinol Metab Diabetes S Afr* 2021;26:382-88, DOI: 10.1080/16089677.2021.1903170
28. Ankita, Stefanickova J, Saxena S, Nim DK, Ahmad K, Mahdi AA, *et al.* Hyperglycemia potentiates the effect of ionic calcium in photoreceptor ellipsoid zone disruption in diabetic retinopathy. *Int Ophthalmol* 2019;39:2237-43.
29. Jud P, Sourij H. Therapeutic options to reduce advanced glycation end products in patients with diabetes mellitus: A review. *Diabetes Res Clin Pract* 2019;148:54-63.
30. Cohrs RJ, Martin T, Ghahramani P, Bidaut L, Higgins PJ, Shahzad A. Translational medicine definition by the European Society for Translational Medicine. *New Horiz Transl Med* 2014;2:86-8.