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ORIGINAL PAPER

Hyperglycemia potentiates the effect of ionic calcium in photoreceptor ellipsoid zone disruption in diabetic retinopathy

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Abstract

Purpose To study the association of serum ionic calcium and glycated hemoglobin (HbA1c) with retinal photoreceptor ellipsoid zone (EZ) disruption in diabetic retinopathy (DR).

Methods This is a tertiary care center-based observational cross-sectional study. Sixty-three consecutive cases, divided into 21 cases each with no diabetic retinopathy, non-proliferative diabetic retinopathy and proliferative diabetic retinopathy were included. Twenty-one healthy controls were also included. Ellipsoid zone disruption was assessed using spectral-domain optical coherence tomography. Serum ionic calcium and HbA1c were measured using standard protocol. Patient data from cases were divided into two groups according to their HbA1c levels: group 1 (HbA1c < 7, $n = 26$) and group 2 (HbA1c > 7, $n = 37$). Data were analyzed statistically.

Results Mean ionic calcium levels in group 1 and group 2 were 1.131 ± 0.073 mmol/dL and 1.170 ± 0.070 mmol/dL, respectively. In group 1, 11 out of 26 had EZ disruption (42.3%). Similarly, in group 2, 29 out

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of 37 had EZ disruption (78.4%). On logistic regression analysis, as compared to group 1, ellipsoid zone disruption was found to be positively associated with serum ionic calcium ($p = 0.01$) in group 2 cases.
Conclusion Increased levels of serum ionic calcium are associated with increased EZ disruption in patients with HbA1c > 7 in DR.

Keywords Diabetic retinopathy · Glycated hemoglobin · Serum calcium · Spectral-domain optical coherence tomography · Ellipsoid zone

Introduction

Diabetes mellitus is one of the leading causes of morbidity worldwide. Globally, 387 million people were living with diabetes in 2014, and the number is set to rise to 592 million within the next 20 years. Chronic hyperglycemia is associated with long-term damage, dysfunction and failure of various organs in diabetes. Effective management of diabetes requires sustained glycemic control over many years to lower the risk of macro- and microvascular complications. Poor glycemic control and duration of diabetes are recognized as major risk factors for the development of complications like nephropathy, neuropathy, coronary heart disease and retinopathy [1–4]. The UK Prospective Diabetes Study has reported a 37% decrease in microvascular disease and a 14% reduction in myocardial infarction (MI) with every 1% reduction in glycated hemoglobin (HbA1c) levels [5].

Diabetic retinopathy (DR) is one of the microvascular complications associated with both type 1 and type 2 diabetes mellitus and is an important cause of preventable blindness in the world [6, 7]. Numerous risk factors have been implicated in the pathogenesis of DR, including poor glycemic control, duration of diabetes, hypertension, increasing age, dyslipidemia, increased serum urea and creatinine [8].

Insulin is an anti-diabetogenic factor involved in glucose metabolism. Insulin receptors are located on both the inner and outer segments of vertebrate photoreceptors [9]. Apoptosis of retinal neurons in diabetic retina during development is blocked by insulin. It may act by reducing ionic calcium influx through L-type voltage-gated ionic calcium channels in the inner segment (IS) of the photoreceptors [10].

On spectral-domain optical coherence tomography (SD-OCT), a hyperreflective band previously thought to represent the photoreceptor inner segment/outer segment (IS/OS) junction, now known as ellipsoid zone (EZ), can be appreciated within the outer retina. The integrity of this layer is a significant predictor of visual acuity in a wide spectrum of surgical and medical retinal diseases [11]. The assessment of the EZ has been utilized to evaluate photoreceptor structure in several macular disorders, including macular hole, macular telangiectasia type 2 and age-related macular degeneration [12–14].

The purpose of this study was to evaluate the association of levels of ionic calcium and HbA1c with photoreceptor EZ disruption in DR.

Methods

The study was performed in accordance to the tenets of the declaration of Helsinki. It was a tertiary care center-based observational cross-sectional study. After obtaining informed voluntary consent and calculating the sample size, 63 consecutive cases were included. Cases were divided into patients with no diabetic retinopathy (No DR; $n = 21$), non-proliferative diabetic retinopathy (NPDR; $n = 21$) and proliferative diabetic retinopathy (PDR; $n = 21$) on the basis of early treatment diabetic retinopathy study (ETDRS) classification [15]. Twenty-one healthy controls presenting for refraction were also included.

Cases with ocular diseases which could affect retinal vascular pathology (hypertensive retinopathy), any previous ophthalmic surgical or laser interventions, end-stage renal disease, cases with signal strength 5 or below on OCT examination and cases taking any mineral supplements or medications causing altered calcium levels in the body were excluded from the study. Slit lamp biomicroscopy and dilated ophthalmoscopic examination were performed. Digital fundus photography and fluorescein angiography were performed using Zeiss fundus camera FF 450 Plus (Carl Zeiss Meditec AG, Jena, Germany). Spectral-domain optical coherence tomography (SD-OCT) [Cirrus HD-OCT Carl Zeiss Meditec, Inc., CA, USA, Model 4000]: macular cube 512 × 128, was done to assess the photoreceptor ellipsoid zone (EZ) disruption. Study subjects underwent spectral-domain optical coherence tomography using macular cube

512 × 128 (Carl Zeiss Meditec Inc., CA, USA). On horizontal and vertical SD-OCT scans, photoreceptor ellipsoid zone disruption was graded into three categories [16]: grade 0: intact photoreceptor ellipsoid zone (EZ); grade 1: focal disruption (photoreceptor EZ disruption indicating subfoveal localized involvement) and grade 2: global disruption (photoreceptor EZ disruption indicating generalized involvement within the macular cube) (Fig. 1).

Blood samples were drawn by aseptic vein puncture from study subjects and transferred into tubes containing 3.89% trisodium citrate (in the ratio of 9:1) for separation of plasma. Serum glycated hemoglobin (HbA1c) was measured on autoanalyzer using standard protocol. Serum ionic calcium was measured by electrolyte analyzer (X1-921C; Caretium medical instruments co. limited, Shenzhen, China) using ion selective method.

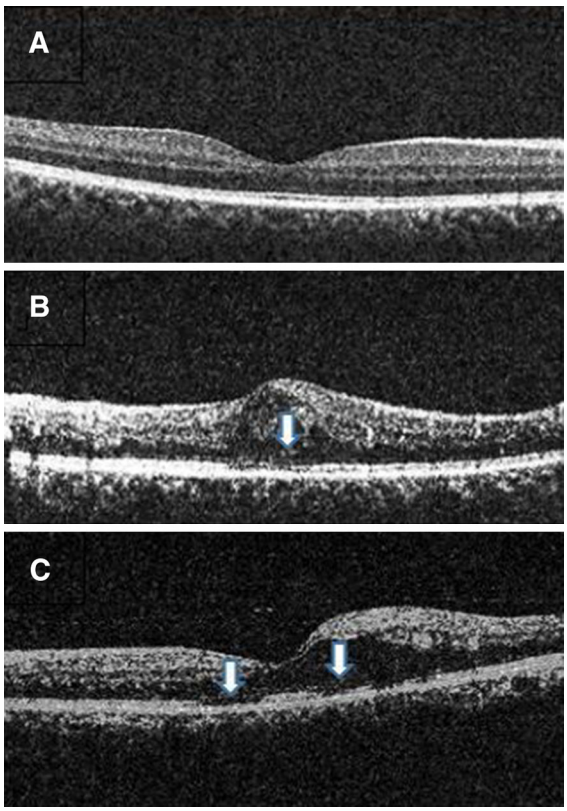

Fig. 1 Spectral-domain optical coherence tomography of macula cross-sectional image illustrating grades of ellipsoid zone disruption. **a** No ellipsoid zone disruption. **b** Focal ellipsoid zone disruption (arrow). **c** Global ellipsoid zone disruption (arrow)

Data were summarized and presented as mean ± SD. Chi-square (χ^2) test analyzed the difference in gender distribution between the groups. Two observers, masked to the case control status of the study subjects, graded the images for disruption of ellipsoid zone (Spearman's correlation = 0.8).

One-way analysis of variance (ANOVA) was done to compare the mean age among the study groups. Independent *t* test was applied to evaluate the differences in means between the controls, No DR, NPDR and PDR. Available data were compiled into two groups according to HbA1c levels of patients, HbA1c < 7, $n = 26$ and HbA1c > 7, $n = 37$. Binary logistic regression was applied for both groups sequentially taking EZ disruption as dependent variable and serum ionic calcium as independent variable. All analyses were performed using SPSS software (window version 21.0) (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, version 21.0. Armonk, NY: IBM Corp).

Results

The mean age was 54.91 ± 7.38 years, 54.05 ± 6.91 years and 50.45 ± 7.39 years, respectively, for the No DR, NPDR and PDR groups. Comparing the mean age of three groups, ANOVA revealed similar age among the groups and was not statistically significant ($F = 1.66$, $p = 0.183$). Chi-square (χ^2) test revealed no significant difference between the study groups ($\chi^2 = 0.54$, $p > 0.05$). On applying t-test, the differences in means for glycated hemoglobin were found to be statistically significant between control versus No DR, control versus NPDR and control versus PDR, and the differences in means for ionic calcium were found to be significantly different only in between control versus NPDR and control versus PDR. Means of serum levels of ionic calcium and HbA1c and frequency of ellipsoid zone disruption among the study groups are summarized in Tables 1 and 2. Mean ionic calcium levels in group 1 and group 2 were 1.131 ± 0.073 mmol/dL and 1.170 ± 0.070 mmol/dL, respectively. In group 1, 11 out of 26 had EZ disruption (42.3%). Similarly, in group 2, 29 out of 37 had EZ disruption (78.4%).

On detailed statistical analysis to evaluate the role of ionic calcium with EZ disruption in the two groups, with HbA1c levels less and more than 7 mg/dl,

Table 1 Illustrating the mean levels of biochemical parameters in the study groups

Group	HbA1c (grams/dL) Mean ± SD	Ionic calcium (mmol/L) Mean ± SD
No DR (n = 21)	6.46 ± .54	1.119 ± .075
NPDR (n = 21)	7.96 ± 1.65	1.167 ± .658
PDR (n = 21)	8.96 ± 1.52	1.176 ± .070

Table 2 Illustrating distribution of ellipsoid zone disruption in the study groups

Group	EZ disruption (n)	
	Absent	Present
No DR (n = 21)	16	5
NPDR (n = 21)	5	16
PDR (n = 21)	2	19

binomial logistic regression was applied between EZ disruption and ionic calcium.

For the first group, with HbA1c < 7, there was no statistically significant association between ionic calcium and EZ disruption ($p = 0.635$). In the second group, with HbA1c > 7, a statistically significant association was found between ionic calcium levels and EZ disruption ($R^2 = 0.183$, $B = 17.89$, $p = 0.01$) (Figs. 2, 3; Table 3).

Discussion

This study evaluates the role of glycated hemoglobin in potentiating the effect of serum ionic calcium in photoreceptor ellipsoid zone (EZ) disruption in DR for the first time. It was found that in patients with poor glycemic control, increased ionic calcium levels are associated with increased EZ disruption.

The calcium ion (Ca^{2+}) is a major messenger for coordinating activity of cells. The functions of ionic calcium are highly compartmentalized in the photoreceptors. In the outer segment (OS), ionic calcium regulates photoreceptor light adaptation in the transduction chain. In the inner segment (IS), ionic calcium regulates cell metabolism, cytoskeletal dynamics, gene expression and cell death [17, 18].

Both the IS and OS are flooded with calcium during darkness. The OS is continuously depolarized by Na^{+}, Mg^{2+} and Ca^{2+} influx through cation selective cGMP-gated (CNG) channels. Ionic calcium exerts a direct control over the cation influx through the CNG channel. Also, ionic calcium has an essential role in modulation of sensitivity of the visual pigment itself.

L-type voltage-gated calcium channels are present in the IS. Many key ionic calcium-dependent cellular enzymes are localized to sub-compartments within the IS. Activation of the inner segment ionic calcium might be expected to trigger a self-regenerative cycle of ionic calcium influx and depolarization which would result in impaired photoreceptor function and cell death.

Sustained increases in intracellular ionic calcium may occur during inherited retinal degenerations, retinal diseases, cell death in humans and animals [19, 20]. It has been suggested that photoreceptor rescue occurs as a result of reduced influx of ionic calcium, lower intracellular levels of ionic calcium and consequent stabilization of ionic calcium homeostasis [21]. In the present study, increased ionic calcium levels, in the presence of increased glycated hemoglobin (HbA1c > 7), resulted in impaired photoreceptor rescue, evident as EZ disruption on SD-OCT.

Elevated levels of ionic calcium have been observed in neurons of diabetic rats [22]. Similar studies on animal models have reported podocyte depletion and apoptosis resulting from up-regulation of calcium flux in diabetic nephropathy [23, 24]. Also, total serum calcium is proposed to be a marker of cardiovascular disease in humans [25].

Our earlier studies have documented that increased EZ disruption was observed with increased severity of DR. Elevated glycated Hb levels were also observed with increased severity of retinopathy [26, 27]. In the

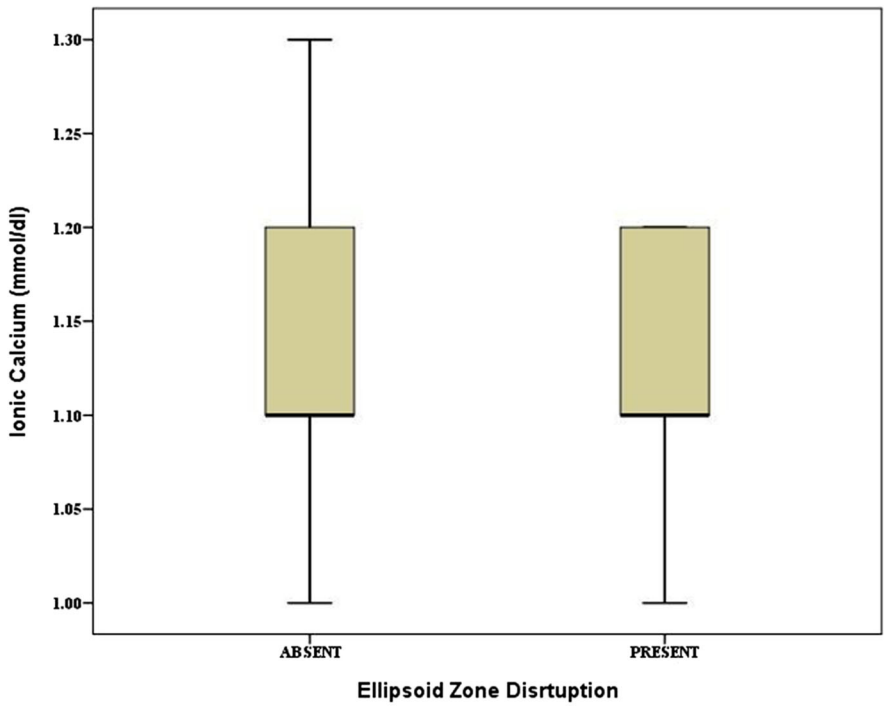

Fig. 2 Box and whisker plot illustrating ionic calcium levels in correlation with ellipsoid zone disruption (0 = disruption absent, 1 = disruption present) in group 1 (glycated hemoglobin < 7)

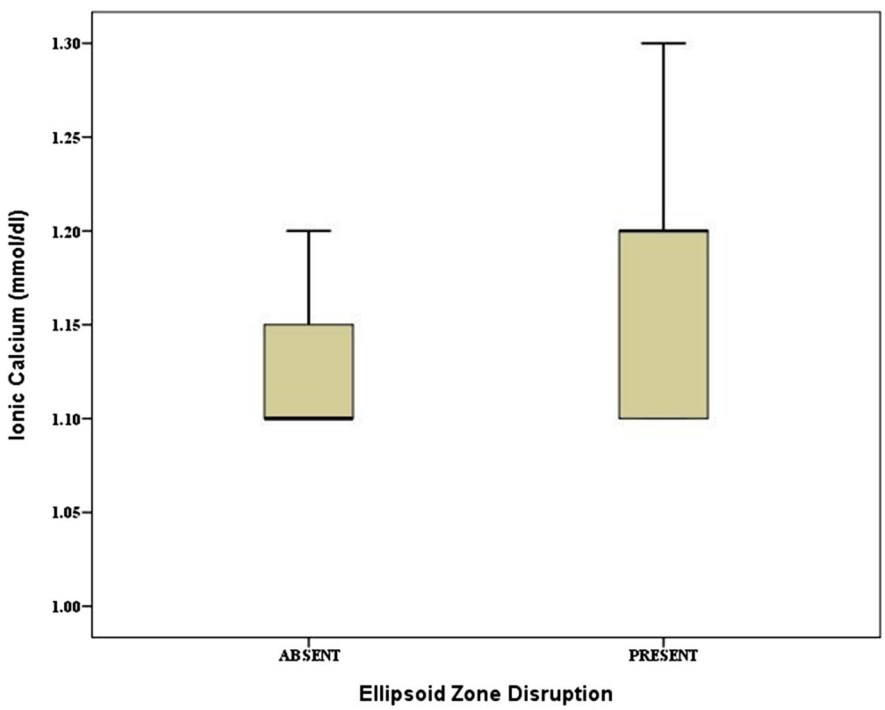

Fig. 3 Box and whisker plot illustrating ionic calcium levels in correlation with ellipsoid zone disruption (0 = disruption absent, 1 = disruption present) in group 2 (glycated hemoglobin > 7)

present study, it was observed that increase in ionic calcium levels leads to increased odds of developing EZ disruption, in association with HbA1c > 7.

It can be concluded that poor glycemic control potentiates the effect of ionic calcium in photoreceptor EZ disruption.

Table 3 Illustrating binomial logistic regression results in the two groups, with glycated hemoglobin less than 7 and more than 7

Group	Odds ratio	R^2	*B* value	SE	*P* value	95% CI
HbA1c < 7	12.59	0.005	2.534	5.343	0.635	0.000–445,224
HbA1c > 7	59,114,481.04	0.183	17.89	7.138	0.012	49.595 to 7.046E+13

Conclusion

The effect of ionic calcium in ellipsoid zone disruption is potentiated by HbA1c > 7.

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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