Case report on two diabetic donor eyes with no retinopathy: Clinicopathological and molecular studies

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We were intrigued to analyze donor eyes of two individuals without retinopathy even after 40 years of type 2 diabetes mellitus. Targeted molecular factors associated with angiogenesis and the key antioxidant enzymes in retinal tissue were analyzed. Accordingly PEDF, Adiponectin and Paraoxonase 2 showed augmented mRNA expression in both the retina with no significant change in VEGF expression. Vitreous showed increased PEDF protein in donor 1 and Adiponectin in donor 2 with no change in VEGF protein. This study highlights the profile of specific molecular factors that contribute to the non-development of diabetic retinopathy changes in these individuals.

Key words: Adiponectin and paraoxonase, diabetic retinopathy, PEDF, VEGF

This study was conducted on the donor retina obtained from two individuals without diabetic retinopathy after 40 years of type 2 diabetes mellitus, with informed consent from their family along with medical records. Diabetic retinopathy is a major microvascular complication which affects 93 million people worldwide and 17 million in India.[1] The pathological features include non-proliferative diabetic

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retinopathy (NPDR), proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) contributed by disruption of blood retinal barrier, oxidative stress and inflammatory changes.[2] Apart from genetic polymorphism, vascular endothelial growth factor (VEGF), pigment epithelium derived factor (PEDF), insulin-like growth factor (IGF), matrix metalloproteinases (MMPs), adiponectin (APN), interleukin 6 and 12 (IL-6, IL-12) are also measured in blood, aqueous and vitreous fluid in patients with and without diabetic retinopathy (DR). VEGF, MMPs, IL-6 are reportedly increased in DR which imparts clues that there could be differentiating markers.[3-6] Although environmental, genetic and metabolic factors contribute to DR, certain endogenous protective factors possibly prevent the vascular complications.[7] The Medalist study has shown that 40% of Insulin dependent diabetes mellitus (IDDM) patients did not present diabetic nephropathy and retinopathy symptoms.[8] With these evidences, we were intrigued to analyze the mRNA expression levels of targeted molecules namely, VEGF, PEDF (Angiogenic), APN, PON2 (antioxidants), LOX, LOXL2, and MMPs (extracellular matrix proteins) [Table 1] in donor retina.

Case Reports

Donor 1 was 83-year-old female, an Asian Indian homemaker, with a past medical history of type 2 diabetes mellitus for 40 years and hypertension for 4 years. She was on medications, Reclide (Gliclazide), Januvia (sitaglitin), Piozone (pioglitazone), Trajenta (Linagliptin) and insulin from 2014. Her last tested HbA1c value was 7.5% as on January 2015. Other medications were Atarax (hydroxyzine), Zolfresh (zolpidem tartrate), Lonazep (Clonazepam), Spasmopriv (Fenoverine), Stablon (Tianeptine), codeine sulphate and Folvite. Her past ocular history reveals posterior sub capsular cataract and nuclear sclerosis which was operated. There was moderate visual impairment in the left eye with nuclear sclerosis and corneal gutatta (OS). We ruled out for glaucoma, high myopia and carotid stenosis, the local factors protective for DR. The systemic complications before demise were upper respiratory tract infection, urinary tract infection, anemia, hyponatremia, breast cancer and acute gastroenteritis. Her right eye was used for this study.

Donor 2 was 89-year-old male, an Asian Indian and an ophthalmologist by profession, with history of type 2 diabetes mellitus for 40 years and 35 years hypertension. He was on the medications namely Euglucon (Glyburide), glynase and Insulin. Other medication includes Cardiovas (Carvedilo), Aldactone (Spironolacton), Lonoxin (digoxin), Rozavel (Rosuvastatin), Ceruvin - A (Clopidogrel), Anti platelets, statins, Cobodex Forte and Coversy (Perindopril). His past ocular history reveals vitreous hemorrhage in his right eye, hard exudates and cataract in left eye since 1986. His recent ocular history shows 6/9 BCVA, IOP 14 mm Hg, cup disc ratio 0.7, with inferior and superior rim, No NFL, attached retina, normal macula, few retinal hemorrhage inferiorly and disruption of IS-OS junction in the left eye which was used for this study. Ocular factors protective for DR were ruled out. Both the donors did not have history of smoking.

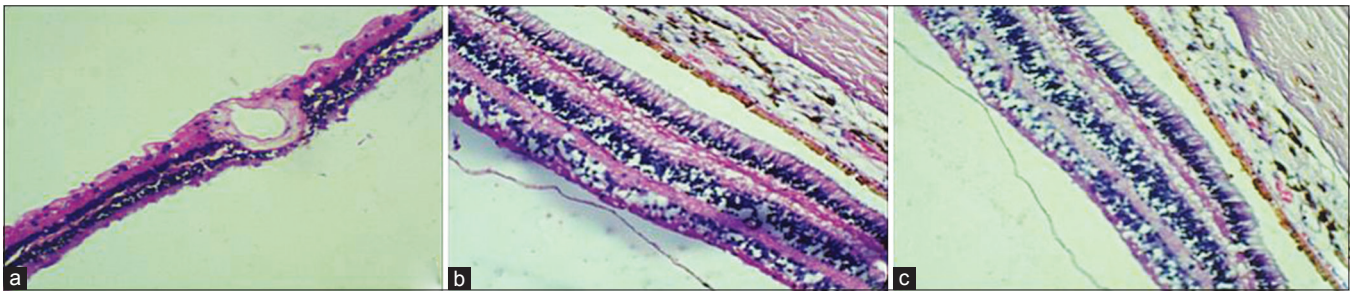

Figure 1: Histopathology evaluation of diabetic donor retina 1 and 2. (a) Right eye of diabetic donor retina 1, (b) right eye of diabetic donor retina 2, (c) left eye of diabetic donor retina 2. The histopathology evaluation of the retina did not show any diabetic retinopathy changes in both the retina

Table 1: Real time PCR primer sequences for the targeted molecules tested

Targeted molecules	Primer Sequence (5'-3')	Targeted molecules	Primer Sequence (5'-3')
PEDF		LOXL2	
Forward	AGGCCCAGAGTCCTGACGGG	Forward	ACTGCAAGCACACGGAGGA
Reverse	CCTTGAAGTGCGCCACACCG	Reverse	AGGTTGAGAGGATGGCTCGA
VEGF		APN	
Forward	CGGTATAAGTCCTGGAGC	Forward	TGGTGAGAAGGGTGAGAA
Reverse	GCCTCGGCTTGTCACATCTG	Reverse	AGATCTTGGTAAAGCGAATG
LOX		PON2	
Forward	ACGGCACTGGCTACTTCCAGTA	Forward	CCACAGCTTTGCACCAGATA
Reverse	TCTGACATCTGCCCTGTATGCT	Reverse	ATGCCATGTGGATTGAATGA
MMP 2		MMP 9	
Forward	CAGGAGGAGAAGGCTGTGTT	Forward	GAGGTGGACCGGATGTTCCC
Reverse	TTAAAGGCGGCATCCACTCG	Reverse	AACTCACTCCGGGACTCAC

Histopathology

Donor eye balls were processed and 5 μm sections were stained with hematoxylin and Eosin. The histopathology evaluation of both the cases did not show any diabetic retinopathy changes like neovascularisation [Fig. 1].

Molecular studies in retina and vitreous

Age- and sex-matched controls were used for comparison [Table 2a and b]. mRNA transcripts of PEDF, LOX and APN of donor 1 was 35, 6 and 40 fold higher in retina, respectively, whereas VEGF, LOXL2 and PON2 were only 2 fold higher compared to control 1. In donor 2, the mRNA transcripts of PEDF, LOX and PON2 was increased by 18, 10 and 8 fold, respectively, Whereas, VEGF was 2 fold as in donor 1 and APN and LOX2 were decreased compared to control 2. MMPs were undetectable in both the donors [Fig. 2]. Vitreous PEDF was 2.5 fold and 5 fold higher in donor 1 compared to macular hole and PDR, respectively but VEGF levels were similar to macular hole vitreous. APN decreased 3 fold compared to PDR vitreous. Donor 2 vitreous PEDF was 3 fold lower compared to macular hole and VEGF levels were nearly similar to PDR vitreous. APN increased by 10 fold and 2 fold compared to macular hole and PDR vitreous, respectively [Table 3]. We observed that the decreased VEGF/PEDF ratio in donor 1 was inhibiting retinal changes which were not observed in donor 2. However, increased APN was compensating for PEDF thereby mitigating retinopathy changes.

Discussion

The anti–angiogenic factors namely, PEDF and adiponectin showed augmented expression in both the donor retina, while the pro-angiogenic factor, VEGF was found to be unaltered revealing "no neovascular changes in the retina" as supported by the retinal pathology. There was a mild retinal capillary BM thickening in donor 1 which may be an early sign of DR development which correlated with the increased expression of LOX. Increase in PON can be protective but oxidative stress was not ruled out. Low VEGF/PEDF ratio or increased APN possibly protected the DR changes in the retina. Thus the study indicates net anti-angiogenic features of the retina which may contribute to the absence of retinopathy changes. The genetic or the environmental factors that mediates this needs further attention. The study limitation is that the observations are based on two cases. Review of more such cases can yield valuable information for conclusion.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Table 2a: Details of the control donor retina used for mRNA expression

Controls	Age and Sex	Systemic Illness	Retina used as control for
1	82/F	NIL	Donor 1
2	89/M	NIL	Donor 2

Table 2b: Details of Vitreous controls used for analysis

Clinical diagnosis	Age and sex	Ocular complications	Systemic Illness	Vitreous used as control for
PDR	53/F	Retinal neo-vascularisation and Vitreous hemorrhage	DM: 8 y HTN: 8 Y	Donor 1
MH	64/F	Full thickness macular hole with cystoid deposits	HTN: 6 y Ischemic Heart disease	Donor 1
PDR	51/M	Retinal neo-vascularisation and Vitreous hemorrhage	DM - 10 yr with HTN	Donor 2
MH	62/M	Macular hole	Nil	Donor 2

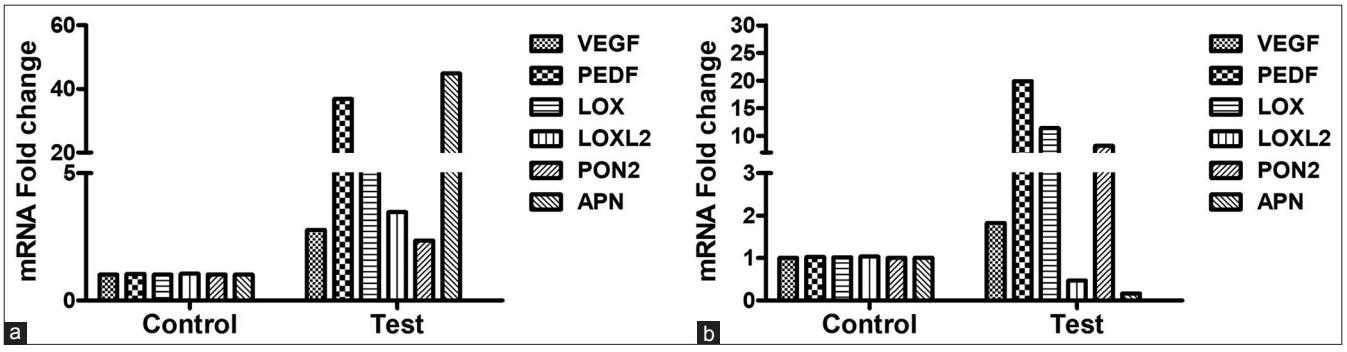

Figure 2: mRNA transcript levels of key proteins in retinal tissue of the diabetic donors 1 and 2 compared to respective controls. (a) donor 1; (b) donor 2; the expression of pigment epithelial derived factor was increased in both the donor's retina and adiponectin expression was increased only in donor 1 retina

Table 3: Vitreous levels of PEDF, APN and VEGF in diabetic donor retina compared to disease controls

	APN (ng/mL)	PEDF (ng/mL)	VEGF (pg/mL)	VEGF/ PEDF ratio
Diabetic donor 1	11.69	**51.6**	12.47	0.24
PDR (53/F)	36.02	10.8	16.07	1.48
MH (64/F)	5.18	20.5	12.35	0.60
Diabetic donor 2	**51.5**	4.85	20.4	4.2
PDR (51/M)	32.3	2.6	14.6	5.6
MH (62/M)	4.81	15.4	12.3	0.79

Conflicts of interest

There are no conflicts of interest.

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