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ORIGINAL PAPER

Systemic oxygen therapy versus oral enalapril for treatment of diabetic macular ischemia: a randomized controlled trial

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Abstract The purpose of this study was to evaluate the structural and functional effects of systemic oxygen therapy and enalapril in patients with diabetic macular ischemia (DMI). This randomized clinical trial consisted of 105 eyes with DMI divided into three groups. Group I received systemic oxygen by face mask at a flow rate of 10 L/min; Group II received 5 mg enalapril daily; and Group III received placebo tablets for 3 months. Best-corrected visual acuity (BCVA), central macular thickness (CMT) measured by optical coherence tomography (OCT), extent of foveal avascular zone (FAZ) on fluorescein angiograms, and electroretinograms (ERG) were obtained at baseline and after 3 and 6 months. Overall, 102 patients completed the study. Baseline characteristics were not significantly different among groups. Significant improvement in BCVA and decrease in CMT and FAZ occurred at months 3 and 6 in oxygen group compared to deterioration in enalapril and control groups (All P values <0.001). ERG parameters were significantly better in oxygen group compared to enalapril group at months 3 and 6 and better than those in control group at month 3. Normobaric oxygen therapy for 3 months in DMI decreased CMT and FAZ and improved BCVA and ERG parameters. Enalapril did not show any favorable effect.

Keywords Diabetic macular ischemia · Enalapril · Ischemic maculopathy · Oxygen therapy

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Trial registration: clinical trials.gov identifier: NCT00899587

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Introduction

Diabetic retinopathy is the most common cause of blindness in adults aged 20–64 years. Visual loss is caused by either proliferative diabetic retinopathy (PDR) and its complications or diabetic maculopathy [1]. Maculopathy may present as edema which is more common or as ischemia. While laser therapy and in recent years pharmacotherapy such as intravitreal corticosteroids and anti-vascular endothelial growth factor (anti-VEGF) agents are the current treatments for diabetic macular edema (DME) [2], there is no effective therapy for diabetic macular ischemia (DMI) [3]. Additionally, it has been shown that the presence of ischemia adversely affects the visual outcomes after treatment for DME [4, 5].

Macular ischemia results from occlusion of foveal capillary network and presents as enlargement and irregularity of the foveal avascular zone (FAZ) leading to disturbance of macular functions. It is postulated that an FAZ larger than 1000 microns is indicative of irreversible visual loss [6].

Angiotensin (Ang) II is a potent vasoconstrictor that binds to Ang II receptors throughout the body including retinal vessels [7]. Current evidence suggests that vasoconstriction induced by Ang II plays an important role in pathophysiology of diabetic nephropathy [8], which is considered as a marker for macular ischemia [9]. Therefore, angiotensin-converting enzyme inhibitors (ACEIs) theoretically might improve macular ischemia. Additionally, they may favorably affect macular ischemia by decreasing systemic blood pressure which is an important risk factor for diabetic retinopathy [10]. Furthermore, ACEIs, through reducing microalbuminuria [11], increase intravascular oncotic pressure which according to the Starling's law may improve macular perfusion.

Increase in arterial oxygen pressure (PaO_2) may improve macular ischemia by several mechanisms: first, by inducing vasoconstriction and reduction of flow, hydrostatic pressure inside the vessels decreases and therefore fluid flow to interstitial space diminishes which in turn results in a decrease in retinal edema and ischemia [12]; second, by increasing oxygen gradient between blood and tissue, oxygen diffusion to the tissue increases which ameliorates ischemia; and third by decreasing VEGF production [13].

Literature is still lacking in higher evidence-based studies looking at the effect of ACEIs or oxygen therapy on DMI. Based on the above-mentioned theories, both of these treatments through various mechanisms could be beneficial in improving macular ischemia and deserve further investigations. Previously, in a pilot study, we observed that systemic oxygen therapy can improve macular structure and function in DMI [14]. In this randomized controlled 3-armed trial, we evaluated the effects of an oral ACEI, enalapril, and oxygen therapy on DMI.

Materials and methods

This study was performed at Labbafinejad medical center, Shahid Beheshti university of Medical Sciences, Tehran, Iran, from 2009 to 2012. Inclusion criteria included patients with type 2 diabetes mellitus and macular ischemia with best-corrected visual acuity (BCVA) $\leq$ 20/30. Macular ischemia was defined as an enlarged FAZ of $\geq$1000 μm in greatest diameter or a broken perifoveal capillary ring at the border of FAZ with a nonperfusion area within 1 disk diameter of the foveal center in the transit phase of fluorescein angiography (FA) [4]. Exclusion criteria included the presence of active PDR, presence of any retinal disease other than diabetic retinopathy, diffuse macular edema, significant media opacities, glaucoma, uveitis, vitreomacular traction, and severe chronic obstructive pulmonary disease as well as a history of intraocular surgery, intravitreal injection, or laser photocoagulation within 3 months of the study. Patients with uncontrolled systemic hypertension (blood pressure more than 140/90 mmHg), fasting blood glucose >200 mg/dL, proteinuria ($\geq$2 + by simple dipstick test), severe anemia (hemoglobin <7 mg/dL), cigarette smoking, and those on ACEIs were also excluded from the study.

Eligible patients were randomized into three groups: Group I (oxygen group) received 100 % oxygen at a flow rate of 10 L/min by face mask for 1 h twice daily for the first month, once daily for the second month, and every other day for the third month; Group II (enalapril group) received enalapril tablet, 5 mg/day for 3 months; and Group III (control) received placebo tablets. Patients were called weekly to ensure that they were using oxygen.

At baseline, complete ophthalmic examination including BCVA (logMAR), ancillary tests including optical coherence tomography (OCT), electroretinography (ERG), and fluorescein angiography (FA) were performed for all patients. Ophthalmic examination was repeated at months 1, 3, and 6 and ancillary tests at months 3 and 6 by a single operator masked to the intervention.

OCT images were obtained using spectral-domain OCT (3D OCT-1000, Topcon Inc, Tokyo, Japan) with raster scan (512 $\times$ 128 axial A-scans) in a 6 $\times$ 6 mm^2 area centered on the fovea. Central macular thickness (CMT) was used for measurement. ERG was recorded by Metrovision ERG system (Metrovision, Pérenchies, France) using corneal electrodes. For each patient, b-wave amplitude (scotopic), b-wave amplitude (rod response), and b-wave amplitude (cone response) were recorded and analyzed. We postulated that eyes with a recent onset macular ischemia are more likely to respond to treatment as opposed to those with chronic ischemia and possibly irreversible structural damage which prevents improvement of macular function. Since in diabetes ischemia is usually not confined to the macula and we were not able to determine the duration of macular ischemia in all patients, we decided to use ERG instead of multifocal ERG (mfERG) to detect any improvement in electrophysiologic activity of the retina. Therefore, even in eyes with chronic macular ischemia which might not show improvement in mfERG responses, it would be possible to detect improvement of total retinal function with ERG.

Retinal angiography was done using HRT2 (Heidelberg engineering Inc, Dossenheim, Germany) with the same adjustments in terms of camera zoom and picture resolution. After acquisition, images were transported to the MATLAB software (R2011b, version 7.13, the Mathworks Inc, US), borders of FAZ were manually outlined, and then size of FAZ was measured in mm^2. The change in the size of the ischemia area (%) at each time point was calculated as follows: post treatment ischemic area—pretreatment ischemic area/pretreatment ischemic area.

The primary outcome measure was BCVA changes at 3 and 6 months. Secondary outcome measures included changes in CMT, size of FAZ, and ERG parameters.

Sample size was calculated for BCVA changes. To have a power of 95 % to detect a difference of 0.2 logMAR in one-by-one comparison of the groups, a standard deviation (SD) of 0.23 logMAR, and a type I error of 0.05, 35 samples were required in each group.

Randomization was performed using random block permutation method according to a computer-generated randomized list with the length varied by 3 and 6. Random allocation sequence was performed by a biostatistician. The study investigators were masked to the details of the randomization.

This study was a single-masked clinical trial. At baseline and at each follow-up examination, an unmasked ophthalmologist completed the physical examination, including slit-lamp examination, tonometry, and funduscopy. BCVA at baseline and at each follow-up, however, was measured by a masked certified optometrist. An ophthalmologist masked to the study groups measured the FAZ size on FA by MATLAB software. OCT, ERG, and FA were performed by a single operator masked to the intervention. Control group received placebo; however, oxygen group was not masked to the intervention.

Statistical analysis

To describe data, we used frequency (percent), mean $\pm$ SD, median, and range. Analysis of Variance (ANOVA), Kruskal–Wallis, and Chi Square tests were applied to evaluate the difference between groups at baseline. To adjust for the baseline, we used ANCOVA. Changes within groups were evaluated with mixed model. Ordinal logistic regression was used to detect the difference between groups regarding the adjusted VA improvement categories. Adjustment for the multiple comparisons was performed by Bonferroni method. *P* values less than 0.05 were considered as statistically significant. All statistical analyses were performed by SPSS software (Version 17.0, SPSS Inc., Chicago, IL).

Results

A total of 105 eyes of 105 patients with DMI were enrolled in the study (35 in each group). There was no significant difference in demographics and baseline values among the study groups (Table 1). During the follow-up period, two eyes developed vitreous hemorrhage (one in the oxygen group and one in the

Table 1 Distributions of baseline characteristics in each treatment group (Mean ± SD)

	Oxygen therapy	Enalapril	Control	*P*
Age (year)	62 ± 7	58 ± 9	58 ± 8	0.129
	61 (49–74)	57 (38–75)	59 (43–74)	
Male/female (Male %)	18/17 (51 %)	23/12 (66 %)	18/17 (51 %)	0.380
FAZ (mm^2)	0.773 ± 0.177	0.775 ± 0.194	0.764 ± 0.173	0.117
	0.758 (0.482–1.191)	0.761 (0.497–1.348)	0.759 (0.498–1.265)	
BCVA (logMAR)	0.57 ± 0.17	0.53 ± 0.19	0.46 ± 0.21	0.051
	0.6 (0.3–0.9)	0.48 (0.18–0.9)	0.4 (0.18–0.9)	
CMT (μm)	367 ± 62	384 ± 50	364 ± 59	0.300
	370 (218–486)	394 (289–459)	346 (283–502)	
ERG parameters (μV)				
b-wave amplitude (scotopic)	281 ± 50	289 ± 49	289 ± 40	0.695
	277 (184–370)	298 (203–359)	307 (204–332)	
b-wave amplitude (rod response)	35.4 ± 8.9	38.5 ± 6.9	35.9 ± 5.5	0.168
	32.3 (19.2–53.3)	39.3 (22.2–49.3)	34.3 (26.7–49.3)	
b-wave amplitude (cone response)	58.8 ± 16	61.4 ± 11.4	63.7 ± 9.8	0.28
	52.5 (37.9–83.3)	62.8 (39.2–77.9)	61.4 (39.4–76.5)	

FAZ foveal avascular zone; *BCVA* best corrected visual acuity; *logMAR* logarithm of minimum angle of resolution; *CMT* central macular thickness; *ERG* elecroretinography

control group) and one neovascular glaucoma (in the enalapril group) at months 3 and 4, respectively.

Within-group analysis revealed that BCVA improved significantly in the oxygen group at 3 and 6 months [-0.09 ± 0.13 ($P < 0.001$) and -0.09 ± 0.10 logMAR ($P = 0.001$), respectively] (Table 2; Fig. 1). However, BCVA deteriorated in both enalapril and control groups at all follow-up visits. Between-group analysis showed that the differences were significant at months 3 and 6 with oxygen group having higher BCVA compared to enalapril and control groups ($P < 0.001$ for both) (Table 2).

At 6 months, 20 % of the eyes in the oxygen group had a visual improvement of ≥2 lines, 54.3 % had an improvement of <2 lines, and 25.7 % had unchanged or worsened BCVA. The corresponding values for the enalapril groups were 0, 0, and 100 % and for the control group were 0, 2.9, and 97.1 %. (Table 3).

Within-group analysis of CMT demonstrated a significant reduction in the oxygen group at 3 and 6 months; -10 ± 14 and -12 ± 13 μ, respectively ($P < 0.001$ for both visits). However, in enalapril and control groups, CMT increased significantly at both follow-up visits (Table 4; Fig. 2). Between-group analysis revealed a significant decrease in CMT in oxygen group at both 3 and 6 months compared to enalapril and control groups ($P < 0.001$ for both).

Within-group analyses of FAZ size revealed significant changes at each measurement compared to baseline in all groups. (Table 5; Fig. 3) However, FAZ decreased in size in oxygen group as opposed to the increase in enalapril and control groups. ($P < 0.001$) (Fig. 3) Multiple comparisons showed that FAZ area in the oxygen group was significantly different from those in other groups at months 3 and 6 ($P < 0.001$ for both).

ERG parameters were compared among the groups before and after treatments (Table 6). All parameters, including scotopic b-wave amplitude, rod response b-wave amplitude, and cone response b-wave amplitude, were significantly different among the groups at both 3 and 6 months. However, multiple comparisons showed that the difference was significant between the oxygen and enalapril groups at 3 and 6 months with oxygen group showing better ERG responses. Such a significant difference was observed in rod response b-wave amplitude between the oxygen and control groups only at the month 3.

Table 2 Mean changes (±SD) and proportions of changes in best-corrected visual acuity (logMAR) in each group at baseline, 1, 3 and 6 months of follow-up

	Oxygen therapy	Enalapril	Control	$P^{\dagger}$	Multiple comparisons‡
Baseline					
Value	0.57 ± 0.17	0.53 ± 0.19	0.46 ± 0.21		
Month 1					
Value	0.56 ± 0.2	0.57 ± 0.21	0.48 ± 0.21	0.103	
Change	−0.01 ± 0.07	0.03 ± 0.07	0.02 ± 0.09		
Change%	−3 ± 15	6 ± 18	8 ± 27		
*P**	0.636	0.051	0.229		
Month 3					
Value	0.48 ± 0.2	0.64 ± 0.23	0.56 ± 0.2	<0.001	(1,2) and (1,3)
Change	−0.09 ± 0.13	0.1 ± 0.1	0.1 ± 0.08		
Change%	−17 ± 29	21 ± 23	27 ± 28		
*P**	<0.001	<0.001	<0.001		
Month 6					
Value	0.48 ± 0.2	0.69 ± 0.21	0.6 ± 0.2	<0.001	(1,2) and (1,3)
Change	−0.09 ± 0.1	0.15 ± 0.11	0.14 ± 0.1		
Change%	−19 ± 22	34 ± 33	42 ± 42		
*P**	0.001	<0.001	<0.001		

logMAR logarithm of minimum angle of resolution

* Based on Mixed model adjusted for multiple comparisons by Bonferroni method

† Adjusted for the baseline value based on Analysis of covariance

‡ Adjusted for the multiple comparisons based on Bonferroni method

Discussion

This study showed that normobaric oxygen therapy for 3 months in eyes with DMI yields better results in terms of retinal function and structure which last for at least 6 months compared to observation and oral enalapril. The favorable functional response after oxygen therapy could be explained, to some extent, by the observed changes in FAZ size and CMT.

Macular ischemia is currently considered as a cause of irreversible visual loss in diabetic patients for which there is no effective treatment [3]. Diabetic microangiopathy with endothelial cell damage results in capillary closure and retinal ischemia on one hand and abnormal vascular permeability and edema on the other hand. In macula both result in visual loss, and by inducing a vicious cycle each exacerbates the other. While DME is currently treated by laser photocoagulation and intravitreal injections of anti-VEGF agents and/or triamcinolone acetonide [2], there is no proven effective treatment for DMI. The presence of macular ischemia is an ominous sign of visual loss and adversely affects the visual outcomes of the eyes treated for DME [4, 5].

According to the Starling's law, the imbalance between hydrostatic and oncotic pressures results in tissue edema [10]. This increases the distance for oxygen to reach FAZ by diffusion and in turn aggravates ischemia. This vicious cycle can be broken by hyperoxia which induces vasoconstriction and decreases flow inside the vessels and hence decreases hydrostatic pressure and fluid flux into the tissue. Additionally, by increasing intravascular oxygen pressure, the gradient of oxygen to diffuse into the tissues increases which improves ischemia. On the other hand, the increased oxygen level also decreases VEGF production, a potent permeability factor and vasodilator, resulting in decreased vascular permeability. Through these two different mechanisms, vascular permeability and vasodilation decrease, further decreasing edema [13]. Increased choroidal oxygenation may improve diabetic choroidopathy and theoretically may improve function of retinal pigment epithelium. Furthermore, supplemental

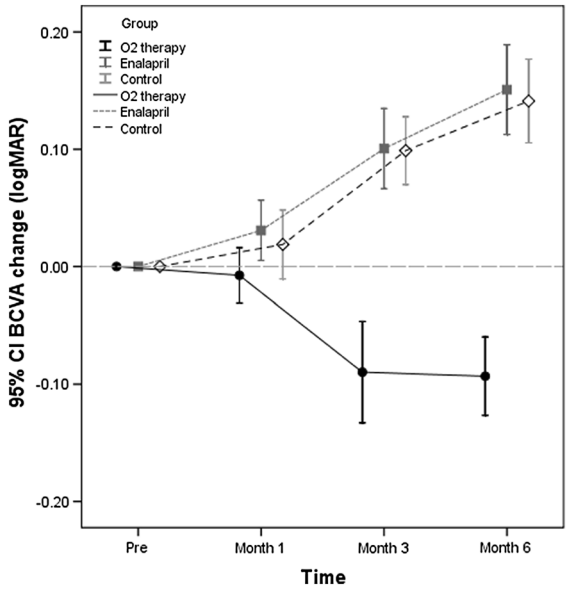

Fig. 1 Mean best-corrected visual acuity (BCVA) changes from *baseline* and 95 % confidence interval (CI) in the three groups at timely visits

oxygen affects many of the components involved in ischemia–reperfusion injury, including polymorphonuclear leukocyte function, endothelial cell adhesion molecule expression, nitric oxide production, nitric oxide synthase expression, cellular energetics, lipid peroxidation, and microvascular blood flow [15]. These mechanisms may explain some of the structural and functional changes observed in oxygen group.

It has been shown that hyperbaric oxygen therapy (HBOT) may improve blood-retinal barrier breakdown and reduce retinal vascular leakage in diabetic rats possibly due to down-regulation of VEGF expression by hyperoxia [16]. Using laser photocoagulation for treatment of DME could also result in increased macular oxygenation through decreasing oxygen consumption by the outer retina and therefore increasing the flux of oxygen from choroid into the inner retina. The favorable effect of vitrectomy on the regression of retinal neovascularization and reduction of macular edema can also be explained by changes in intraocular oxygen pressure gradient [12].

Several studies have evaluated the effects of oxygen therapy, either normobaric [17] or hyperbaric [18, 19], for macular edema of various etiologies. In two studies on macular edema due to retinal vein occlusion, HBOT has been reported to improve VA that persisted in some patients up to 6 months after treatment [18, 19]. Similar beneficial effect has been reported in a case of radiation-induced macular ischemia treated by HBOT [20]. In a pilot study on DME unresponsive to macular laser photocoagulation, Nguyen et al. showed that continuous normobaric oxygen therapy (4 L/min) for 3 months reduced CMT in all cases; however, VA improvement occurred in less than half of the eyes. These beneficial effects diminished after cessation of oxygen therapy [17]. In another study, administration of 100 % oxygen in patients with mild non-proliferative diabetic retinopathy resulted in significant increase in contrast sensitivity, whereas no change occurred in the control group [21].

Table 3 Distribution of cases with 2 line improvement in visual acuity in each group at 1, 3 and 6 month of follow-ups

	Oxygen therapy (%)	Enalapril (%)	Control (%)	*P**
Month 1				
Unchanged or worse	26 (74.3)	32 (91.4)	29 (82.9)	0.241
<2	9 (25.7)	3 (8.6)	6 (17.1)	
≥2	0 (0.0)	0 (0.0)	0 (0.0)	
Month 3				
Unchanged or worse	12 (34.3)	33 (94.3)	34 (97.1)	<0.001
<2	15 (42.9)	2 (5.7)	1 (2.9)	
≥2	8 (22.9)	0 (0.0)	0 (0.0)	
Month 6				
Unchanged or worse	9 (25.7)	35 (100.0)	34 (97.1)	<0.001
<2	19 (54.3)	0 (0.0)	1 (2.9)	
≥2	7 (20.0)	0 (0.0)	0 (0.0)	

* Adjusted for the baseline values, based on ordinal logistic regression

Table 4 Mean changes (±SD) and proportions of changes in central macular thickness (μm) in each group at baseline, 3 and 6 months of follow-up

	Oxygen therapy	Enalapril	Control	$P^{\dagger}$	Multiple comparisons‡
Baseline					
Value	367 ± 62	384 ± 50	364 ± 59		
Month 3					
Value	357 ± 67	391 ± 51	372 ± 62	<0.001	(1,2) and (1,3)
Change	−10 ± 14	7 ± 6	9 ± 10		
Change%	−3 ± 4	2 ± 1	2 ± 2		
*P**	<0.001	<0.001	<0.001		
Month 6					
Value	355 ± 67	398 ± 52	376 ± 63	<0.001	(1,2) and (1,3)
Change	−12 ± 13	14 ± 7	13 ± 11		
Change%	−4 ± 4	4 ± 2	3 ± 3		
*P**	<0.001	<0.001	<0.001		

* Based on Mixed model adjusted for multiple comparisons by Bonferroni method

† Adjusted for the baseline value based on analysis of covariance

‡ Adjusted for the multiple comparisons based on Bonferroni method

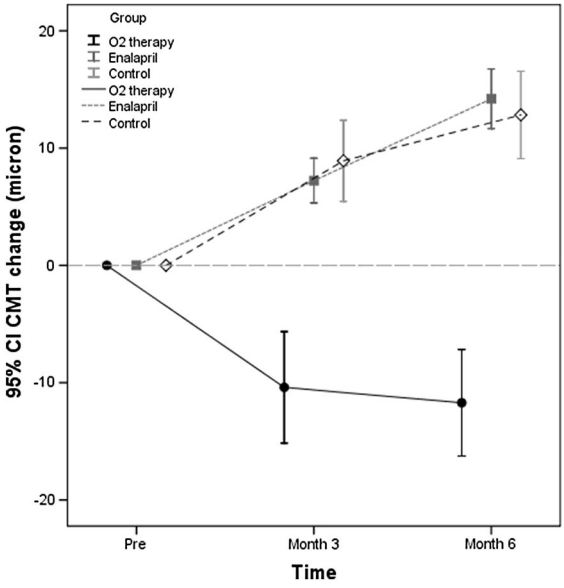

Fig. 2 Mean central macular thickness (CMT) changes from *baseline* and 95 % confidence interval (CI) in the three groups at timely visits

Considering the above-mentioned mechanisms and lack of an effective treatment for DMI, we evaluated the effects of supplemental oxygen therapy on this devastating disorder. In a pilot study, we previously showed the beneficial effects of oxygen therapy on retinal structure and function in DMI [14].

In our study, BCVA increased in oxygen group and decreased in enalapril and control group which shows the natural course of the disease if left untreated. However, BCVA did not increase in about 25 % of patients in oxygen group. It can be postulated that chronic ischemia with irreversible changes may respond less to oxygen therapy compared to recent onset ischemia; although we were not able determine the duration of ischemia before the study in most cases.

Oxygen group showed better ERG responses compared to enalapril and control groups that could be indicative of improvement in retinal function by increasing tissue oxygenation. Pavlidis et al., in a study evaluating the effect of hypobaric hypoxia at high attitude on macular structure and function, showed that multifocal ERG responses decreased significantly after high attitude exposure and returned to normal after descent [22].

In order to detect the effects of treatment on macular perfusion, we measured FAZ size before and after interventions. We noticed a reduction in FAZ size after treatment in the oxygen group as opposed to enalapril and control groups which showed a significant increase in FAZ size during the study.

Table 5 Mean changes (±SD) and proportions of changes in foveal avascular zone (mm^2) in each group at baseline, 3 and 6 months of follow-up

	Oxygen therapy	Enalapril	Control	$P^{\dagger}$	Multiple comparisons‡
Baseline					
Value	0.773 ± 0.177	0.775 ± 0.194	0.764 ± 0.173		
Month 3					
Value	0.753 ± 0.176	0.783 ± 0.189	0.773 ± 0.174	<0.001	(1,2) and (1,3)
Change	−0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01		
Change%	−2.68 ± 1.63	1.28 ± 1.68	1.22 ± 1.31		
*P**	0.000	0.002	0.002		
Month 6					
Value	0.759 ± 0.181	0.785 ± 0.191	0.779 ± 0.171	<0.001	(1,2) and (1,3)
Change	−0.01 ± 0.02	0.01 ± 0.02	0.01 ± 0.01		
Change%	−1.96 ± 2.14	1.55 ± 2.15	2 ± 1.69		
*P**	0.000	0.004	0.001		

* Based on Mixed model adjusted for multiple comparisons by Bonferroni method

† Adjusted for the baseline value based on Analysis of covariance

‡ Adjusted for the multiple comparisons based on Bonferroni method

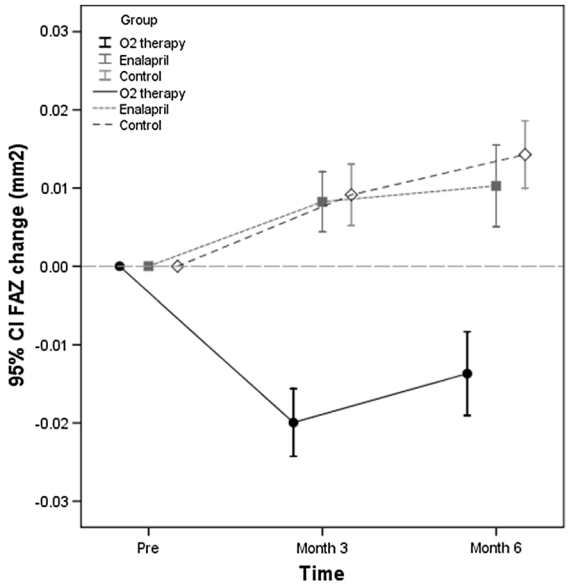

Fig. 3 Mean foveal avascular zone (FAZ) changes from baseline and 95 % confidence interval (CI) in the three groups at timely visits

One of the major complications of diabetes is diabetic nephropathy in which vasoconstriction, mediated by Ang II, plays an important role [8]. The renin–angiotensin–aldosterone system (RAAS) is a hormonal cascade that begins with the synthesis of renin by renal tissue in response to decreased perfusion pressure. This results in formation of Ang I from angiotensinogen. Ang I is then hydrolyzed by angiotensin-converting enzyme to form Ang II. Ang II acts by binding to specific receptors, AT1 and AT2. RAAS is important in the regulation of blood pressure, renal function, tissue perfusion, and extracellular volume. Dysregulation of RAAS is involved in the pathogenesis of renal disorders. ACEIs and angiotensin-receptor blockers are used to treat hypertension and renal disease [23]. Ang II receptors have been found in ocular tissues, and the retina has the highest levels. AT1 receptors are present in Muller cells and the blood vessels of the inner nuclear layer and are more frequent in diabetics than non-diabetics [7]. Ang II can enhance vascular permeability by upregulation of VEGF [24]. It also has powerful angiogenic actions and is associated with overexpression of angiogenic factors such as VEGF, and thus it is involved in the development of retinal neovascularization [25]. On the other hand, ACEIs and angiotensin-receptor blockers may reduce the progression of diabetic retinopathy [26, 27]. It has been shown that treatment with either an ACEI or an AT1 blocker can normalize retinal blood flow in diabetic rats [28]. Furthermore, in

Table 6 Mean changes (±SD) and proportions of improvement in electroretinographic parameters (μV) in each group at baseline, 3 and 6 months of follow-up

		O_2 therapy	Enalapril	Control	$P^{\dagger}$	Multiple comparisons‡
b-wave amplitude (scotopic)						
Baseline	Value	281 ± 50	289 ± 49	289 ± 40		
Month 3	Value	286 ± 51	288 ± 48	288 ± 39	0.020	(1,2)
	Change%	2 ± 5	0 ± 4	0 ± 3		
	*P**	0.002	0.519	0.570		
Month 6	Value	284 ± 54	287 ± 46	286 ± 38	0.031	(1,2)
	Change%	1 ± 3	0 ± 4	−1 ± 3		
	*P**	0.233	0.463	0.072		
b-wave amplitude (rod response)						
Baseline	Value	35.4 ± 8.9	38.5 ± 6.9	35.9 ± 5.5		
Month 3	Value	36.7 ± 9.4	36.6 ± 7	35.3 ± 6	<0.001	(1,2) and (1,3)
	Change%	4 ± 7	−5 ± 8	−1 ± 10		
	*P**	0.006	<0.001	0.309		
Month 6	Value	37.5 ± 9.6	36.1 ± 7.2	36 ± 5.6	<0.001	(1,2)
	Change%	6 ± 7	−6 ± 10	1 ± 15		
	*P**	0.002	0.001	0.891		
b-wave amplitude (cone response)						
Baseline	Value	58.8 ± 16	61.4 ± 11.4	63.7 ± 9.8		
Month 3	Value	60.2 ± 16.2	60.2 ± 11.3	63.6 ± 9.2	0.015	(1,2)
	Change%	3 ± 6	−2 ± 6	0 ± 7		
	*P**	0.008	0.020	0.890		
Month 6	Value	59.7 ± 15.9	59.7 ± 11.6	62.8 ± 9.7	0.022	(1,2)
	Change%	2 ± 5	−2 ± 8	−1 ± 6		
	*P**	0.222	0.017	0.315		

* Based on Mixed model adjusted for multiple comparisons by Bonferroni method

† Adjusted for the baseline value based on analysis of covariance

‡ Adjusted for the multiple comparisons based on Bonferroni method

diabetic patients, intraocular levels of Ang II and VEGF are elevated and correlate with the severity of retinopathy [29].

Despite the above-mentioned mechanisms, we did not observe any beneficial effect in the enalapril group. However, the result of this study could not undervalue the importance of RAAS in the development and progression of diabetic retinopathy. It could be due to incomplete blockade of RAAS with enalapril. Addition of angiotensin-receptor blockers or increase in dosage and duration of treatment with ACEIs may result in therapeutic response.

There are some limitations in our study. We did not measure blood vessel diameter, flow velocity, and retinal oxygen before and after oxygen therapy to correlate them with the outcomes. Additionally, oxygen was administered for only 3 months which seems short for a lifelong disease process.

In summary, the current study showed that normobaric oxygen therapy for 3 months has beneficial anatomical and functional effects in patients with DMI that last for a minimum of 3 months after cessation of therapy. Since DMI is usually associated with DME, addition of DME treatments such as anti-VEGF and corticosteroids to oxygen therapy may enhance therapeutic effects which can be the subject of future studies. Oral ACEI, enalapril, did not result in any favorable outcomes in our study. However, the effects of other ACEI agents with higher doses and angiotensin-receptor blockers need to be evaluated in future studies.

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Compliance with ethical standards

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

Ethical approval All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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