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Evaluation of New Therapeutic Combination Efficacy in Diabetic Macular Edema

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□ ABSTRACT □

The increasing number of individuals with diabetes suggests that diabetic retinopathy DR is a major contributor to vision loss. The initial disease is characterized by increased vascular permeability due to a breakdown in the blood-retinal barrier BRB, causing macular edema DME, with a progressive vascular occlusion and retinal neovascularization which are secondary to ischemia and oxidative stress. Laser photocoagulation and vitrectomy only target advanced stages of disease. However, despite laser treatment, patients with DME experienced gradual loss of vision. Intravitreal triamcinolone IVTA reduces the breakdown of BRB and down-regulates the production of vascular endothelial growth factor VEGF. IVTA may moderately but temporarily improves visual acuity in cases of DME. Agents that attenuate VEGF action such as bevacizumab are expected to reduce permeability and neovascularization. Intravitrealbevacizumab IVB reduces macular edema secondary to central retinal vein occlusion, vascular permeability and fibrovascular proliferationin. Calcium dobesilate CD is a potent antioxidant, slows vascular proliferation. The antioxidant activity of oral calcium dobesilate OCD is a result of its hydroquinone structure which interacts with toxic-free radicals induced by ischemia. Tomography OCT and electro retinographyERG are high-performance techniques to diagnose diabetic retinopathy and macular edema. OCT imaging is analogous to B-scan ultrasound imaging, except that it uses infrared light reflections instead of ultrasound and allows quantitative measurements of retinal thickness in DME. Electro retinography ERG is the neurophysiological test used in order to measure electric changes that happen in the retina after a light stimulus; and is able to detect retinal dysfunction prior to the onset of visible retinopathy. In our study, we have found that combination between laser photocoagulation, anti-edematous (triamcinolone), anti VEGF (bevacizumab) and antioxidant (calcium dobesilate) is effectual in decreasing diabetic morphological lesions and electric retinal dysfunctions manifestedin DME, and that this synergetic cotreatments has improved patients' visual acuity and life quality.

Keywords: diabetic retinopathy**,** macular edema, photocoagulation**,** bevacizumab, triamcinolone**,** calcium dobesilate**,** tomography, electroretinography.

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تقييم فعالية مشاركة علاجية جديدة في وذمة اللطخة الصفراء السكرية

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□ ملخّص □

يتبين من العدد المتزايد للمصابين بداء السكري بأن اعتلال الشبكية السكري سبب أعظمي في فقدان البصر. المميزات البدئية للمرض هي زيادة النفوذية الوعائية ناجمة عن انكسار الحاجز الشبكي الدموي مسببا" وذمة اللطخة مترافق مع انسداد وتوعي وعائي جديد ثانويين لنقص التروية والأكسدة. التخثير بالليزر و قطع الزجاجي يستهدفان فقط الحالات المتقدمة للمرض، لكن رغم العلاج بالليزر فإن مرضى الوذمة السكرية يشتكون من نقص القدرة البصرية التدريجي. يحمي حقن التريامسينولون في الزجاجي الحاجز الشبكي الدموي وينظم إنتاج العامل المنمي للأوعية كما يحسّن القدرة البصرية بشكل معتدل لكن مؤقت في حالات وذمة اللطخة. تنقص العقاقير التي تثبط العامل المنمي للأوعية VEGF كالبيفاسيزوماب (أفاستين) النفوذية و التوعي الجديد. يؤدي حقن الأفاستين في الزجاجي إلى نقص وذمة اللطخة الثانوية للانسداد الوريدي الشبكي المركزي كما ينقص النفوذية الوعائية وتكاثر الألياف الوعائية. دوبيسيلات الكالسيوم، مضاد أكسدة قوي، يبطئ التكاثر الوعائي. تعود الآلية المضادة للأكسدة لدوبيسيلات الكالسيوم الفموي إلى بنية الهيدروكينون التي تتفاعل مع الجذور الحرة السامة الناجمة عن نقص التروية الشبكية. التوموغرافي و تخطيط الشبكية الكهربائي تقنيات عالية لتشخيص اعتلال الشبكية و وذمة اللطخة الصفراء السكريين. التصوير بالتوموغرافي مشابه للتصوير بالأمواج فوق الصوتية إلا أنه يستخدم انعكاس الأشعة تحت الحمراء مما يسمح بقياس كمي لسماكة الشبكية في وذمة اللطخة. تخطيط الشبكية الكهربائي هو اختبار عصبي فيزيولوجي يستعمل في قياس التغيرات الكهربية التالية لتحريض ضوئي على الشبكية، ويمكن بواسطته تحري اعتلال الشبكية الوظيفي قبل ظهور الآفات المرئية. في بحثنا، اكتشفنا أن المشاركة بين التخثير بالليزر ومضاد للوذمة (التريامسينولون) ومضاد لل VEGF (البيفاسيزوماب) و مضاد للأكسدة (الدوبيسيلات) فعالة في إنقاص الآفات التشريحية و الوظيفية وذمة اللطخة الصفراء السكرية، و أن هذه المشاركة التآزرية حسّنت حدة البصر و مستوى حياة المريض.

الكلمات المفتاحية: اعتلال الشبكية السكرية، وذمة اللطخة الصفراء السكرية، التخثير، البيفاسيزوماب، التريامسينولون، دوبيسيلات الكالسيوم، التوموغرافي، تخطيط الشبكية الكهربائي.

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INTRODUCTION:

Diabetes is now considered an epidemic with an increasing number of affected patients raising to more than 180 million people worldwide[1]. This epidemic is largely due to the increase in type II diabetic patients showing resistance to insulin by contrast to the insulin deficiency in type I diabetes. As a consequence, diabetic retinopathy DR the first cause of blindness before the age of 50 will continue to be an increasing contributor to vision loss and associated functional impairment even in developed countries[2].DR is one of the main diabetic complications resulting from poor glucose control and chronic hyperglycemia. It is characterized by micro vascular complications in the retina with neuronal loss leading to blindness. Vascular changes are associated to vascular permeability due to a breakdown in the blood-retinal barrier BRBwhich causes macular edema DME[3]. Neovascularization, the growth of abnormal retinal blood vessels, is occurring at very advanced stages of the pathology leading to proliferative diabetic retinopathy PDR. This blood vessel growth is a response to the tissue retinal ischemia in an attempt to increase the oxygen and metabolite supplies to the retina.In cases of DR, diagnosis is traditionally basedonophthalmoscopically visible retinal changes, and frequently, the findings are confirmed by fluorescein angiography whichisauseful method in detecting early alterations of the BRB, capillary closure, and microaneurysm formation[4]. Recently, optical coherence tomography OCT has revolutionized the way clinicians can assess the retina. OCT allows non-invasive*in-vivo* visualization of retinal anatomy that is rapid andeasy to acquire, and well-tolerated by patients. The most sensitive parameter related toDME is precise measurement of macular thickness[5].Electroretinography ERG is a physiological test used to study the retinal function in order to find out the neurodegenerative changes. Changes in the ERG may be due to an impairment of any of the retinal cell types: photoreceptors (a-wave ERG), and amacrine, bipolar, and, mainly, Müller cells (b-wave ERG). Moreover, oscillatory potentials are likely to be due to inner retinal neurotransmission. Electroretinographic and psychophysical studies have demonstrated that retinal function is altered in diabetic eyes and is related to duration of disease. ERG is able to detect retinal dysfunction prior to the onset of visible retinopathy[6].

Early detection of retinopathy in individuals with diabetes is critical in preventing visual loss. The best preventive care appears to be the tight control of blood glucose. However, when the disease is already in advanced stage, classic treatments include laser photocoagulation LP and vitrectomy. LP is likely to reduce energy consumption by decreasing the retinal surface and it could as well facilitate oxygen and metabolite flows to the retinal tissue. Successful laser treatment reduces visual losses but has limited effects on improving macular edema and visual acuity[7]. Different pathways were found to affect the BRB and thus macular edema. Intravitreal triamcinolone acetonide IVTA was for instance shown to reduce the breakdown of BRB by down-regulating the production of vascular endothelial growth factor VEGF. IVTA improves moderately visual acuity in cases of DME refractory to laser treatment, but it generally offers only short-term improvements[7,8]. Experimental studies have suggested that their mechanism of action could be inhibition of mineralocorticoid receptors[9].Recent studies have instead focused on the different molecules to trap and neutralize directly VEGF such as antibodies. These therapeutic agents can not only act on the BRBrupture but also neovascularization, the hallmarks of DME and PDR[10]. For these therapies, bevacizumab, a VEGF antibody, has been reported to be very efficient with no major events[11].Finally, the last therapeutic strategy for DR and DME has been the use of antioxidant molecules[12,13].Among such

antioxidants, calcium dobesilate has been used for more than 40 years[14]. Calcium dobesilate,by blocking hydroxyl radicals, improves diabetic endothelial dysfunction, reduces apoptosis, and slows vascular cell proliferation[14].In the present study, we investigated potential combinations of treatments to improve DME. These combinations included LP, anti VEGF, glucocorticoid and antioxidant.

OBJECTIVES OF THE STUDY

This study is designed to compare the best correctedvisual acuity,the anatomicchanges and the electricalameliorations in type II diabetic patients with DMEat 7 months of primary treatment with LP and of different cotreatments(IVTA, IVB, OCD).OCT and ERGare used to quantify the retinal thickness RT and the functional changes,respectively, pre and post cotreatments. We used these rigorous techniques as evaluation tools for this comparative study to assess DME before and after special combination treatments in order to identify the potential benefits of a novel therapy designed to limit the risk of macular edema, and progressive vision loss while also reducing or eliminating the risk factors of this disease.

MATERIALS AND METHODS

Subjects: we used mixed gender adults volunteers patients with type II diabetes and diabetic retinopathy. We reviewed the clinical records of 71 consecutive patients (108 eyes) with DME. Mean age of 66±8(58-74) years were included in this analysis.

Selection Criteria: inclusion and exclusion for subjects enrolled in the study

Inclusion Criteria

- Signed written consent- Between the ages of 58 and 74, inclusive- Any race or gender
- Diagnosis of DMEin at least 1 eye Documented on fundoscopy and angiography
- Able to understand and comply with the requirements of the trial
- No conditions that limit the view to the fundus (e.g.vitreous hemorrhage, cataract)
- Subjects must be available for a minimum trial duration of approximately 10 months
- Subjects or eyes must not meet any of the exclusion criteria

Exclusion Criteria: Any of the following excluded a subject from the trial:

- Currently enrolled in an ophthalmic clinical trial- Eyes with concomitant macular disorders- Subjects with significant ocular lens opacities causing vision decrease

- Subjects with amblyopia- Subjects with optic nerve disease (neuropathy, atrophy, papilledema), intraocular inflammation; diagnosis of glaucoma; previous intraocular surgery apart from cataract extraction, chronic use of topical ocular steroid medications, previous retinal laser treatment (e.g. focal or scatter photocoagulation.

- Subjects who have received any previous experimental procedure in either eye or the use of any drug or treatment within 30 daysprior to enrolling in the trial

- Subjects who have had intraocular surgery in trial eye within 6 months prior to enrolling in the trial

Study Procedure: All patients underwent a complete ocular and systemic assessment once they consented for the study. The assessment was performed by the primary investigator before they were randomized into groups (Table 1).

Table (1): Study design and protocol of our experience

SCREENING	DIVISION IN GROUPS	VISIT 1: WEAK 1 EXAM	VISIT 2: WEAK 2	VISIT 3: 1 MONTH LATER	VISIT 4: 6 MONTHS LATER
Signs informed consent ↓ Meets inclusion/exclusion criteria? ↓ Subject enrolled or Not enrolled ↓ 71 patients (108 eyes) with DR	Randomized 3 groups: 36 eyes/group ↓ 1.bevacizumab 2.bevacizumab+triamcinolone 3.bevacizumab+triamcinolone +calcium dobesilate	EXAM (Parameters Measurements) ↓ Visual acuity OCT ERG	Laser Photocoagulation	Visual acuity OCT ERG Cotreatment Procedure ↓ Intravitreal and Oral Treatments According to Groups	EXAM ↓ Visual acuity OCT ERG

1. Pre-Treatment Parameters Measurements

1.1. Visual acuity VA

Visual acuity of both eyes was tested with the standardretro illuminated Snellen chart. All patients underwent subjective refraction by oneoptometrist.Parameters of visual acuity used in this study are shown in Table 2.

Table (2): Parameters of visual acuity used in our experience

Visual Acuity Parameters	>5/10	1/10-5/10	<1/10
Assessment	more than 5 line	±	less than one line

1.2. Fundus examination

Fundus examination was done using 78 Dioptre lens on slit lamp biomicroscopy and binocular indirect ophthalmoscopy.Diabetic Macular Edema was classified as mild, moderate andsevere based on the International Clinical DiabeticMacular Edema Disease Severity Scale[15].

Table (3): Diabetic Macular Edema Disease Severity Scale

Mild diabetic macular edema	Moderate diabetic macular edema	Severe diabetic macular edema
Hard exudates in posterior pole but distant from the center of the macula	Retinal thickening or hard exudatesapproaching the center of the macula but not involving the center	Retinal thickening or hard exudates involving the enter of the macula

1.3. Fluorescein Angiography FA

Fluorescein angiogramswere performed on a Heidelberg scanning laser ophthalmoscope (Heidelberg Engineering, Heidelberg, Germany).Some diabetic features

could be assessed better with fluorescein angiograms. In the early-mid phase of the FA, the foveal avascular zone, capillary loss, capillary dilatation, arteriolar and retinal pigment epithelium abnormalities were assessed. Fluorescein leakage, source and cystoid changes were graded during the late FA phase.

1.4. Optical Coherence Tomography OCT

OCT examinations (Figure. 1) were performed using the OCT 3000 scanner (Carl Zeiss Ophthalmic System Inc). All OCT examinations were done by the same operator, and all scans were done with a scan length of 6 mm. The foveal thickness was defined as the distance between the vitreoretinal interface and the retinal pigment epithelium in the center of the fovea.OCT is a high-resolution technique that permits cross-sectionalvisualization of the retinal structure in which the time delays of lightreflected from different depths within the retina are located by meansof low-coherence interferometry. It utilizes light to image tissue using low coherence interferometry. OCT offers several advantages over slit lamp biomicroscopy and FA. OCT produces cross sectional images of the macula allowing objective evaluation of macular thickness and evaluation of the vitreomacular interface including diffuse retinal thickening, cystoid macular edema, posterior hyaloidal traction, serous retinal detachment and tractional retinal detachment.

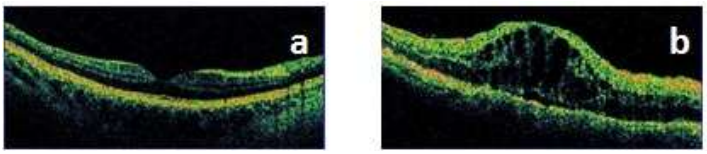


Figure (1):Tomography of control eye (a) and eye with macular edema (b)

Table (4): OCT qualitativeinterpretation[16]

Hyperreflectivity	Hyporeflectivity	Shadow effect
Hard exudates	Intraretinal edema	Hemorrhages
Cotton wool spots	Exudative retinal detachment Cystoid macular edema	Exudates Retinal vessels

Table (5): OCT parameters used in our study

OCT Morphological Changes	Edema non detected	Intraretinal edema	Exudative retinal detachment	Cystoid macular edema
OCT Retinal Thickness µm	Normal 170±20	Mild edema 190-219	Moderate edema 220-250	Severe edema >250

1.5. Electroretinography ERG

The eye was tested after pupil dilation (1% tropicamide, 2.5% phenylephrine). All patients had pupil diameters greater than 6 mm. Silver/nylon fiber electrodes (DTL, Laird Technologies, Sauquoit Inc. Scranton, USA) were used. The active electrode was placed over the middle third of the lower eyelid of each eye. The forehead served as reference and the ipsilateral ear served as ground. The ISCEV-ERG GF program which is an integrated part of the system (Roland Consult, Electrophysiological Diagnostic Systems, Wiesbaden, Germany) was used to record standard ERGs. For oscillatory potentials, patients were light adapted for 10 min with a background light of $15cdm^{-2}$. The light intensity of the flash was $3cdsm^{-2}$.The high-pass filter was set at 75–100 Hz, and the low pass filter set at 300 Hz. Light adapted responses included a single white flash.For flickers ERGs, the intensity of flashes was $3cdsm^{-2}$ with a background luminance of $30cdm^{-2}$.Flashes were presented at a rate of approximately 30 stimuli per second (30 Hz) with 0.5sbetween flashes.Thirty

recordings were averaged with an interstimulus interval of 30 seconds.After 10 min of light adaptation, a photopic flash response of 10cdsm^{-2} light intensity with a background light of 25cdm^{-2} was used. Ten recordings were averaged with an interstimulus interval of 30 seconds.

Table (6): Electroretinography parameters

Electroretinography	Oscillatory Potentials OP2	Photopic b-wave	30 Hz Flickers b-wave
Flash Intensity	3 cd/m^2	10 cdsm^{-2}	3 cdsm^{-2}
Background Light	15 cd/m^2 sec	25 cdsm^{-2}	30 cdm^{-2}

2. Treatment Procedure

2.1. Laser photocoagulation

Patients were properly positioned on a stable chair withthe chin rested on the slit lamp that was mounted with alaser wavelength, Carl Zeiss Visulas 532S laser system. Topical anaesthetic, 5% proparacainehydrochloride was instilled in the eye whichneeded to be lasered.Patients were underwent grid or focal photocoagulation depending on the intensity of lesions. The laser settings were 50 micronspotsize, duration of 0.1 seconds and appropriate power started from 50 mW and stepped up till it burned the retina with light gray burn. The number of laser burn given was based on the severity of DR (range: 20 - 200 laser burns and 500 µm away from the center of the fovea). Only one session of laser (either focal or grid laser) was given to each patient. The procedure was done by ophthalmologists. Patients were followed up during one month post laser and no other treatment was given during that period. Then eyes were divided in 3 equal groups, each was treated monthly with one of these combinations:

2.5 mg of bevacizumab

2.5 mg of bevacizumab and triamcinolone acetonide4 mg in a volume of 0.1 ml

2.5 mg of bevacizumab and triamcinolone acetonide4 mg in a volume of 0.1 ml and calcium dobesilate 500 mg/3 times/day

This treatment protocol lasted six consecutive months (one injection every month).

2.2.Intravitreal injections

Intravitreal injections were carried out under sterile conditions in the operation room.Topical chloramphenicol four times a day was prescribed one day prior to procedure.Theselectedeye had been prepared in a standardfashion using 5% povidone/iodine. An eyelid speculum was used to stabilize the eyelids, and the injection of 2.5mg of bevacizumab, 4 mg triamcinolone (Kenalog) was performed 3.5 to 4 mm posterior to the limbus, through the inferotemporal pars plana with a 30-gauge needle under topical anesthesia or subconjunctivallidocaine. After the injection, IOP and retinal artery perfusion were checked, and patients were instructed to administer topical antibiotics for 7 days. Every patient was intravitreally injected once per month for six consecutive months.1500 mg of calcium dobesilate per day in three divided doses for 6 months.

3. Post-Treatment Parameters Measurements

Patients were followed-up after one month post LP and at 7 months post LP and combination medications. The similar step of visual acuity, fundoscopy, ERG and OCT assessment as pretreatment measurement was done.

4. Statistical analysis

Statistical analysis of the results was performed by a two-way analysis of variance with the Bonferroni's test (Prism5) for all measurements. A probability ($p<0.05$) was taken as a criterion for statistical significance.

Ways to minimize study error

The following steps were taken to reduce errors while conducting the study:

i. Patients were selected strictly based on the inclusionand exclusion criteria.

ii. Randomization of patients.

iii. LP and intravitreal injections were performedby experienced ophthalmologist who was masked topatient's identity.

RESULTS

Mean age, gender, duration of Diabetes Mellitus were shown in Table 7.Detailed values of each group and percentages were shown in Table 8.

Table (7): Demographic data

Number of patients	Number of eyes	Mean age Year	gender		Duration of diabetes Year
			Male	Female	
71	108	66 ±8(58-74)	41	30	16±4

Table (8):Detailed results and percentage of values

VISUAL ACUITY 36 eyes/group	bevacizumab				bevacizumab+ triamcinolone				bevacizumab+ triamcinolone+ calcium dobesilate			
	>5/10	1/10-5/10	<1/10	No changes	>5/10	1/10-5/10	<1/10	No changes	>5/10	1/10-5/10	<1/10	No changes
Before treatment	4	22	10	0	4	21	11	0	4	23	9	0
Laser+1M	7	13	8	8	7	14	8	7	7	14	7	8
Laser+7M	10	18	4	4	15	11	7	3	21	9	4	2

VISUAL ACUITY 36 eyes/group **percentage**	bevacizumab				bevacizumab+ triamcinolone				bevacizumab+ triamcinolone+ calcium dobesilate			
	>5/10	1/10-5/10	<1/10	No changes	>5/10	1/10-5/10	<1/10	No changes	>5/10	1/10-5/10	<1/10	No changes
Before treatment	11.11	61.11	27.77	0	11.11	58.33	30.55	0	11.11	63.88	25	0
Laser+1M	19.44	36.11	22.22	22.22	19.44	38.88	22.22	19.44	19.44	38.88	19.44	22.22
Laser+7M	27.77	50	11.11	11.11	41.66	30.55	19.44	8.33	58.33	25	11.11	5.55

TOMOGRAPHY Morphological Changes 36 eyes/group	bevacizumab				bevacizumab+ triamcinolone				bevacizumab+ triamcinolone+ calcium dobesilate			
	Edema non detected	Intraretinal edema	Exudative retinal detachment	Cystoid macular edema	Edema non detected	Intraretinal edema	Exudative retinal detachment	Cystoid macular edema	Edema non detected	Intraretinal edema	Exudative retinal detachment	Cystoid macular edema
Before treatment	4	18	7	7	5	18	8	5	4	17	9	6
Laser+1M	7	13	11	5	8	14	10	4	7	13	12	4
Laser+7M	10	10	12	4	16	12	7	1	22	10	4	0

TOMOGRAPHY Morphological Changes 36 eyes/group **percentage**	bevacizumab				bevacizumab+ triamcinolone				bevacizumab+ triamcinolone+ calcium dobesilate			
	Edema non detected	Intraretinal edema	Exudative retinal detachment	Cystoid macular edema	Edema non detected	Intraretinal edema	Exudative retinal detachment	Cystoid macular edema	Edema non detected	Intraretinal edema	Exudative retinal detachment	Cystoid macular edema
Before treatment	11.11	50	19.44	19.44	13.88	50	22.22	13.88	11.11	47.22	25	16.66
Laser+1M	19.44	36.11	30.55	13.88	22.22	38.88	27.77	11.11	19.44	36.11	33.33	11.11
Laser+7M	27.77	27.77	33.33	11.11	44.44	33.33	19.44	2.77	61.11	27.77	11.11	0

TOMOGRAPHY Retinal Thickness µm 36 eyes/group	bevacizumab				bevacizumab+ triamcinolone				bevacizumab+ triamcinolone+ calcium dobesilate			
	Normal 170±20	Mild edema 190-219	Moderate edema 220-250	Severe edema >250	Normal 170±20	Mild edema 190-219	Moderate edema 220-250	Severe edema >250	Normal 170±20	Mild edema 190-219	Moderate edema 220-250	Severe edema >250
Before treatment	3	23	5	5	4	22	4	6	3	24	5	4
Laser+1M	8	13	12	3	8	15	11	2	9	14	11	2
Laser+7M	12	10	7	7	19	6	8	3	25	6	3	2

TOMOGRAPHY Retinal Thickness µm 36 eyes/group **percentage**	bevacizumab				bevacizumab+ triamcinolone				bevacizumab+ triamcinolone+ calcium dobesilate			
	Normal 170±20	Mild Edema 190-219	Moderate Edema 220-250	Severe edema >250	Normal 170±20	Mild Edema 190-219	Moderate Edema 220-250	Severe edema >250	Normal 170±20	Mild Edema 190-219	Moderate Edema 220-250	Severe edema >250
Before treatment	8.33	63.88	13.88	13.88	11.11	61.11	11.11	16.66	8.33	66.66	13.88	11.11
Laser+1M	22.22	36.11	33.33	8.33	22.22	41.66	30.55	5.56	25	38.89	30.55	5.55
Laser+7M	33.33	27.77	19.44	19.44	52.77	16.66	22.22	8.33	69.44	16.66	8.33	5.55

ELECTRORETINOGRAPHY 36 eyes/group	bevacizumab			bevacizumab+ triamcinolone			bevacizumab+ triamcinolone+ calcium dobesilate		
	Oscillatory Potentials	Photopic	Flickers	Oscillatory Potentials	Photopic	Flickers	Oscillatory Potentials	Photopic	Flickers
Before treatment	67.85	81.78	8.12	63.3	79.95	9.61	66.8	82.43	10.58
Laser+1M	97.34	88.47	11.1	95.85	89.66	12.1	98.55	86.76	11.86
Laser+7M	132.12	130.64	14.32	177.32	153.58	21.33	207.23	187.75	44.3

Comparison between groups after LP

VA After one month of LP, the number of eyes with visual acuity more than 5 lines in Snellen Chart increased similarly in all groups (AV, AV+TR, AV+TR+CD). Figure 2A

OCT: Morphological changes Using OCT showed that after one month post laser, the number of eyes with "Edema Non Detected" increased equally in all groups also. Figure 2B

OCT: Retinal thickness Moreover, retinal thickness diminished neutrally (reduction of retinal swelling, Normal=170±20 µm) in all groups. Figure 2C

ERG Concerning retinal function, the amplitude of OP2, b-wave Photopic and Flicker ERGs increased equally in all groups also (raise: OP2 31.6%, Photopic 7%, Flickers 20.3%). Figures 2D-F

Comparison between groups after LP and cotreatment course

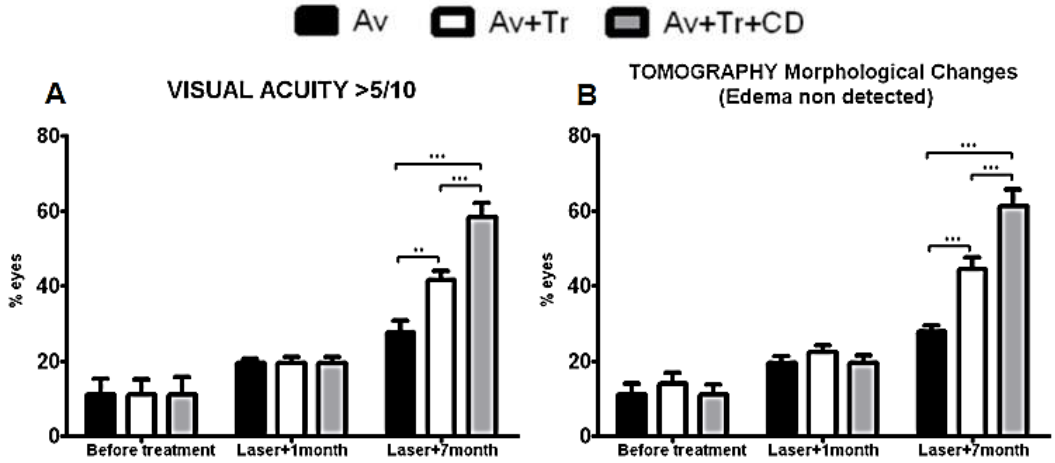
The number of improved eyes of the groups increases progressively as seen below:
AV ⟶AV+TR ⟶AV+TR+CD

VA After 7 months of LP and cotreatments, the number of eyes with visual acuity more than 5 lines in Snellen Chart increased in all cotreatments groups. The best outcome was seen in the AV+TR+CD group with significant total towards the AV and AV+TR groups ($p<0.001$). Figure 2A

OCT: Morphological changes With OCT we recognize that after 7 months post laser and co-medications, the number of eyes with "Edema Non Detected" increased also in all groups with significant difference of ($p<0.001$) between (AV, AV+TR+CD) groups and between (AV+TR, AV+TR+CD) groups. Figure 2B

OCT: Retinal thickness likewise, retinal thickness diminished in all groups with expressive decrease in AV+TR+CD group with variance ($p<0.001$) compared to AV groupand ($p<0.05$) compared to AV+TR group. Figure 2C

ERG As for retinal electric transmission, AV alone moderately increased the amplitude of OP2, b-wave Photopic and Flickers ERGs. Whereas AV+TR+CD cotreatment increased all ERGs; and all amplitudes (OP2, Photopic, Flickers) were significantly high in comparison with the AV group($p<0.001$) and the AV+TR group ($p<0.001$). Figure 2D-F



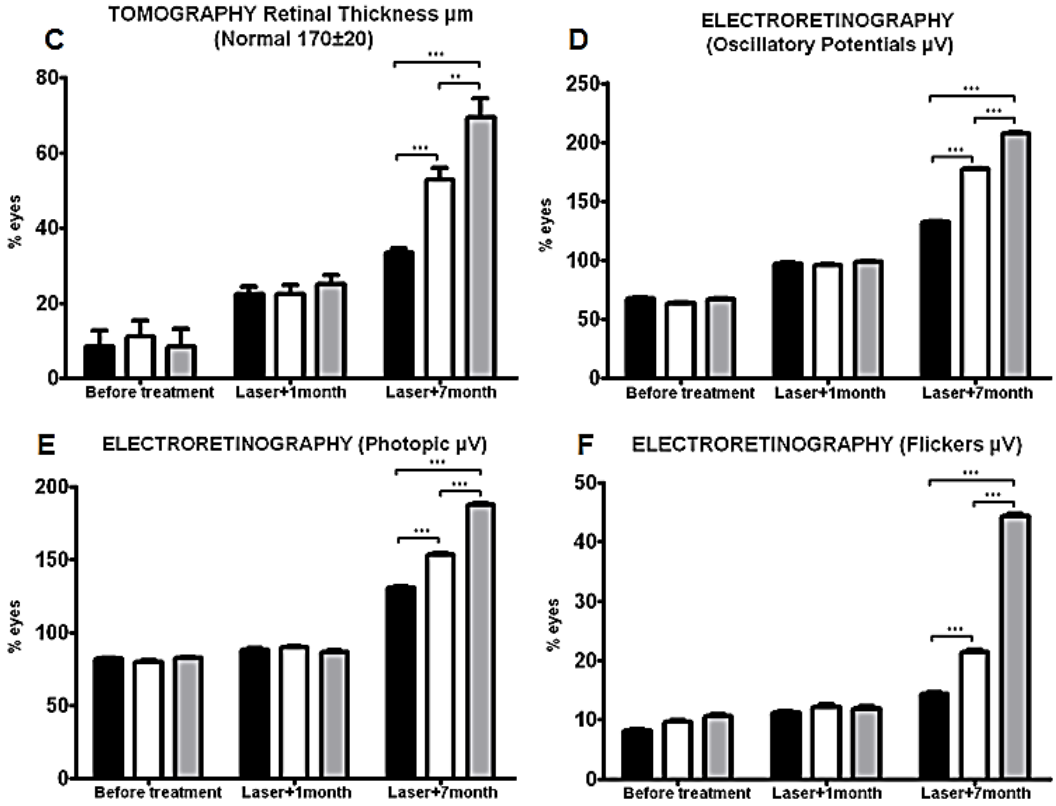


Figure (2): Comparison between groups after LP and comparison between groups after LP and cotreatment course

Comparison of VA, OCT, ERGs before and after treatments for each group

Since the ill eyes were equally divided in 3 groups and the number of eyes of each group before treatment with visual acuity VA >5 lines, with normal retinal thickness and with ERG amplitudes approximately convergent, we decided to compare the outcomes of VA, OCT and ERG before and after laser and cotreatments.

VA The comparison of percentages of eyes with VA more than 5 lines in Snellen chart after laser and post cotreatment for each of the three groups is shown in Figure 3A. The mean difference for VA within the groups at baseline, after laser and at 7 months post cotreatment was statistically significant: $p< 0.05$ for the AV group, $p<0.001$ for the AV+TR group, $p< 0.001$ for the AV+TR+CD group with the best outcome for AV+TR+CD. Figure 3A

OCT: Retinal thickness The comparison of percentages of eyes with normal retinal thickness after laser and post cotreatment for each of the three groups is shown in Figure 3B. Similarly, the difference was statistically significant: $p < 0.001$ for the AV group, $p< 0.001$ for the AV+TR group, $p< 0.001$ for the AV+TR+CD group with the best outcome for AV+TR+CD. Figure 3B

ERG Comparing the amplitude OP2 of Oscillatory potentials after laser and post cotreatment for each group, we found the mean difference was significant: $p< 0.001$ for the AV group, $p< 0.001$ for the AV+TR group, $p< 0.001$ for the AV+TR+CD group. The best outcome was for AV+TR+CD. Figure 3C

Comparing the amplitude of photopic b-wave after laser and post cotreatment for each group, the mean difference was significant: $p< 0.001$ for the AV group, $p< 0.001$ for

the AV+TR group, p<0.001 for the AV+TR+CD group. The best outcome was for AV+TR+CD. Figure 3D

Concerning the amplitude of flicker b-wave after laser and post cotreatment for each group, the mean difference was significant: p< 0.001 for the AV group, p< 0.001 for the AV+TR group, p< 0.001 for the AV+TR+CD group. The best outcome was for AV+TR+CD. Figure 3E

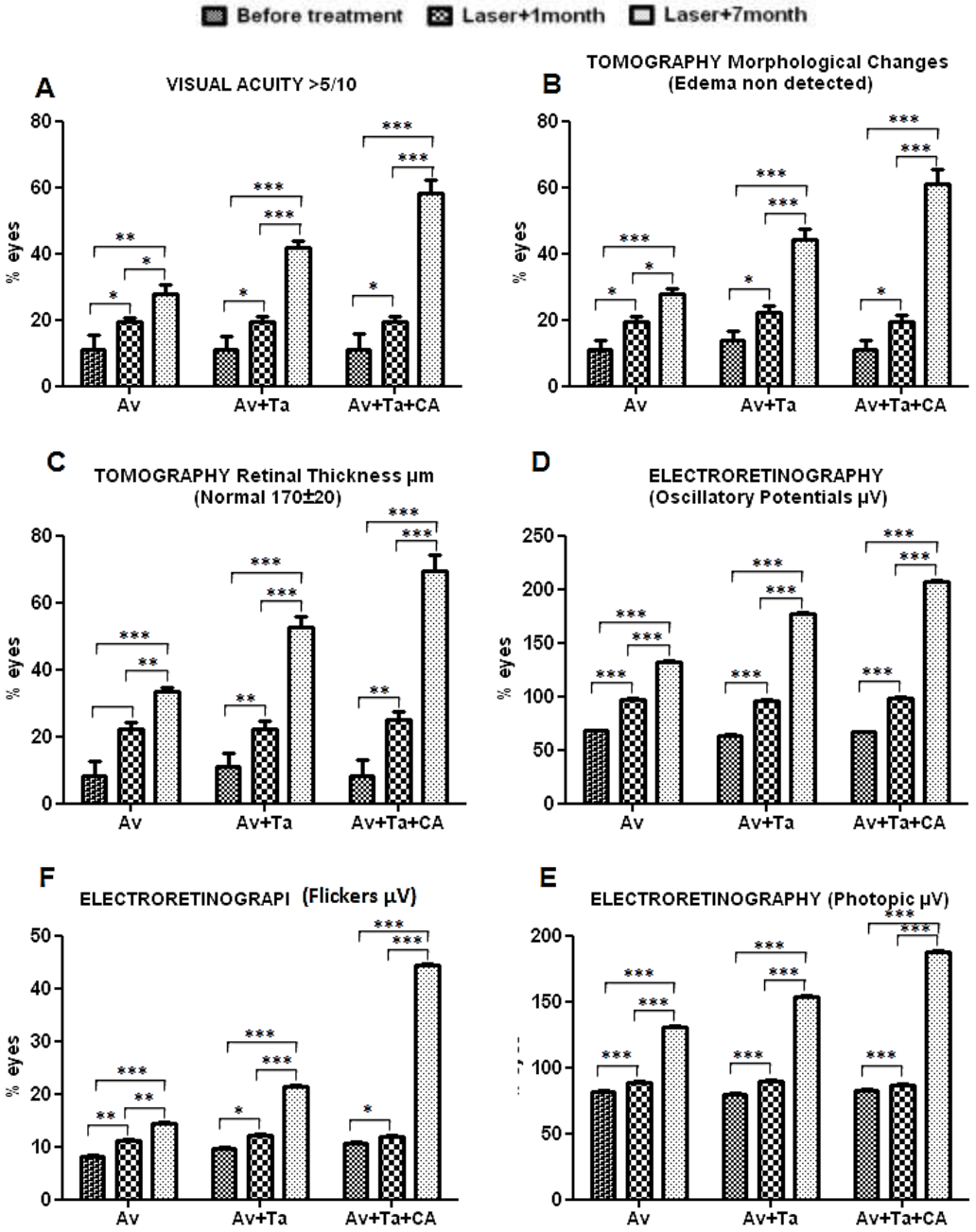


Figure (3): Comparison of VA, OCT, ERGs before and after treatments for each group

It is obvious that the most expressive difference was in the AV+TR+CD group. This confirm that combination of multiple molecules with synergetic mechanisms of actions (laser, anti VEGF, antiedematous, antioxident) against DME is stronger and more efficacious in diabetic retinopathy recovery. The cotreatment "AV+TR+CD" was the best

in increasing the improvement characters of retinopathic eyes. Our results indicate that cotreatment "AV+TR+CD" may have a beneficial effect on visual acuity, macular thickness, independent of the type of macular edema that is present and on oscillatory potentials, photopic and flicker ERG amplitudes. Therefore, in the future this new cotreatment modality could replace or complement focal/grid LP. Furthermore, LP should be used to consolidate the results obtained with this combination therapy and decrease the need for long-term intravitreal reinjections.

DISCUSSION

DR is the major cause of blindness in persons less than 70 years of age in developed countries and is the leading cause of blind registration in the working population. According to the World Health Organization WHO, DR is thought to be responsible for approximately 5% of all blindness worldwide. DR is a chronic progressive sight-threatening ocular condition that is associated with small vascular changes in the retinal circulation. There is substantial evidence that control over metabolic factors (hyperglycemia, hyperlipidemia, hypertension) can effectively prevent the development and progression of DR/ DME. However, many patients fail to achieve or maintain optimal levels of metabolic control. For such patients, early detection and timely treatment of DR remains the standard of care. Although they are effective, sight-saving interventions, laser photocoagulation therapy, and vitrectomy are invasive, associated with destructive side effects, and only treat the late stages of disease. A number of pharmacological agents that could slow the progression of DR/DME in earlier stages are now being tested. It is likely that one or more of these pharmacological interventions, or possibly combinations thereof, will be effective in reducing the progression of DR and DME and the associated vision loss.

Laser photocoagulation is used to treat both PDR and DME. The goal of macular laser photocoagulation for DME is to limit vascular leakage through a series of focal laser burns at leaking microaneurysms or grid laser burns in regions of diffuse breakdown of the blood retinal barrier. The rationale of panretinal photocoagulation for PDR is to ablate ischemic areas of the peripheral retina and thereby reduce the induction of angiogenic growth factors. Results of the Diabetic Retinopathy Study demonstrated that panretinal photocoagulation effectively reduces the risk of vision loss in a majority (60%) of patients with PDR[17,18]. Although the Early Treatment Diabetic Retinopathy Study (ETDRS)[19] demonstrated that immediate focal photocoagulation reduced moderate visual loss by 50%, 12% of treated eyes still lost ≥15 ETDRS letters at the 3-year follow-up interval. Approximately 40% of treated eyes that had retinal thickening involving the center of the macula at baseline still had thickening involving the center at 12 months. Furthermore, only 3% of laser-treated eyes experienced a gain of ≥3 lines of vision. This suggests that a distinct subgroup of eyes exists with DME resistant to conventional laser photocoagulation.

VEGF has been shown to be an endothelial cell-specific mitogen and an angiogenic inducer in a variety of *in vitro* and *in vivo* models[10]. VEGF has been demonstrated to increase retinal vessel permeability byincreasing the phosphorylation of tight junction proteins. Also, hypoxia has been shown to be a major inducer of VEGF gene transcription[20]. Recent work has found elevated levels of VEGF in ocular fluids of patients with PDR and DME [21,22,23].Avastin is a monoclonal antibody that exerts its effect by attaching to and inhibiting the action of VEGF, preventing the formation and growth of new blood vessels. It was the first such therapy, designed to inhibit

angiogenesis, that was approved by the US Food and Drug Administration (FDA)[10]. Avastin has been shown to improve visual acuity in patients with DME refractory to laser therapy which is the equivalent of a two lines (10-letter) gain on the ETDRS scale. In patients who have not yet undergone laser therapy, Avastinsignificantly improved visual acuity versus laser therapy in trials lasting up to one year[24,25, 26]. The absolute benefit increase ranged from 15% to 19% over 6 to 52 weeks. The diabetic retinopathy clinical research network (DRCRnet)[27] performed a randomized clinical trial of eyes with DME and found that in eyes receiving pan-retinal photocoagulation, that the addition of intravitrealranibizumab (recombinant humanized monoclonal antibody fragment with specificity for all isoforms of human VEGF) injections is associated with better VA and decreased macular edema by 14 weeks.Intravitreal triamcinolone acetonide (IVTA) has been shown experimentally to reduce the breakdown of blood retinal barrier[28]. It constitutes a newer, less destructive treatment modality in the management of DME. Two previous studies of primary IVTA in DME[29] have shown improvement on visual acuity as well as central macular thickness. Massin*et. al.* compared the use of IVTA as an adjunctive therapy in DME eyes which failed laser treatment where it effectively reduced the macular thickening[29]. Jonas *et. al.* in 2003 reported in their prospective, interventional, clinical case series study, the visual acuity had significantly improved with IVTA[29].Kang *et al.*[30] randomized 86 eyes with diffuse DME to receive either IVTA or IVTA followed by grid laser. They found significant improvement in VA and central macular thickness in the IVTA plus laser group after 3 weeks. Lam *et al.*[31] performed a randomized controlled trial of 111 patients with DME randomized to grid laser photocoagulation, 4 mg IVTA, or 4 mg IVTA combined with sequential grid laser approximately 1 month later. They showed that IVTA combined with laser produced a greater reduction in central macular thickness compared to laser alone or IVTA alone. Recently, Gillies *et al.*[32] published a prospective, double-masked, randomized, placebo-controlled study of 84 eyes with DME. At 24 months, there was doubling of improvement in vision by 10 or more letters in 15 (36%) of 42 eyes treated with IVTA plus laser compared with 7 (17%) of 42 eyes treated with laser only.Calcium dobesilate is one of the oldest drugs used in the treatment of diabetic retinopathy. The first reports concerning its usage appeared in the late 1960th[33]. Its mechanisms of action are related to its reactivity as a reducing agent including lowering of reactive oxygen species-induced capillary permeability, enhancement of nitric oxide synthase activity in endothelial cells, influence on apoptosis in vessel and other tissues, effects on expression of cellular adhesion molecules, angiogenesis inhibition, reduction of retinal albumin leakage, influence on platelets and blood viscosity, influence on plasma levels of endothelin[34,35,36]. Calcium dobesilate still remains the only angioprotective agent that reduces the progression of diabetic retinopathy[37,38].Basic research provides indications about the involvement of fibroblast growth factor FGF in DR [39,40,41]. It has also been described that the levels of fibric growth factor FGF are higher in the vitreous from patients with DR in which neovascularization and DMEare evident [42]. Involvement of FGF, a well characterized inflammatory, angiogenic and vascular leakage-inducing protein [43,44] supports that intravitreal FGF inhibitors may be ideal candidates to treat DR by counteracting FGF over-expression. It has been revealed that dobesilate is the most effective member of a new family of efficient FGF inhibitors [45] that is effective when administered locally in inflammatory/angiogenesis-related conditions [46,47]. Pedro Cuevas*et al.* 2012 [48] reported that after intravitreal administration of dobesilate, normalized histological structure of the retina and a considerable improvement of visual acuity were observed.In

our study we found that combination between laser photocoagulation and therapeutic molecules (IVB, IVTA, OCD) showed to be more efficacious and synergetic, as well, side effects and adverse events were less manifest. As soon as DME is diagnosed, the ophthalmologist should start the cotreatment (bevacizumab+triamcinolone+cadobesilate) because it is effective from the beginning of the fundoscopical symptoms contrary to laser photocoagulation which is usually used in late stages of DR when dangerous complications such as hemorrhages are present. It is evident that this medication synergy increases the converse efficacy against VEGF and expressively reduces the new vessels formation. Moreover, this combination increases the antioxidant effect against laser burns and the sun irradiation.Limitation of this present study was a short duration of follow-up. A longer period of follow-up, at least over 12 months would give more value especially to arrive a treatment recommendation and able to assess the side effect of long-term therapy.

CONCLUSION

Laser photocoagulation combined with Intravitrealbevacizumab, triamcinolone and oral calcium dobesilate seem to provide stability or improvement in visual acuity, tomography, and electroretinography in DME at 7 months. This combination therapy demonstrates good outcome comparable to laser photocoagulation alone. Follow-up is still short; however, the results appear promising. Evaluation in a multicenter randomized controlled clinical trial with longer follow-up is needed.

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