AIM OF THE WORK

The work is aiming to quantify photoreceptor outer segment (PROS) length in patients with diabetic macular edema (DME) using spectral domain optical coherence tomography (OCT), and describe the correlation between PROS length and visual acuity before and after intravitreal triamcinolone acetonide injection.

SUBJECTS AND METHODS

I. Subjects

The study included 30 eyes of diabetic patients who were selected from the Ophthalmology Outpatient Clinic at Alexandria Main University Hospital.

Inclusion criteria

A. Slit lamp examination: Media clarity no external eye infection (including conjunctivitis, meibomianitis, and significant blepharitis) and patient cooperation sufficient to inject the intravitreal triamcinolone acetonide to obtain adequate funds photographs and OCT.

B. Visual acuity assessment: best corrected visual acuity ranged from 3 /60 and 6\18 (Snellen chart), and 0. 05 to 0.33 by decimal chart.

C. **Intraocular pressure measurement:** intraocular pressure measured by Goldman applantion tonometery not more than 15 mmHg.

D. Slit lamp fundus biomicroscopic examination by contact and non contact lens: Presence of diffuse clinically significant macular oedeoma which is defined by a zone, or zones of retinal thickening, one disc area or larger, any part of which is within one disc diameter of the center of the macula on

E. Fluoresceien angiography: presence of diffuse fluorescein leakage involving the center of the macula on fluorescein angiography.

F .OCT: presence of diabetic macular edema the retinal thickness above 350 microns

Exclusion criteria

a) Presence of an ocular condition (other than diabetes) that might produce macular edema or alter visual acuity during the course of the study as:

1. Media opacities
2. Vein occlusion
3. Uveitis or other ocular inflammatory disease
4. Glaucoma
5. Irvine-Gass syndrome
6. Vitreomacular interface disease
7. Vitreous hemorrhage

b) History of major ocular surgery (including cataract extraction, vitrectomy, scleral buckle, any intraocular surgery, etc.)

Informed Consent

Informed consent was obtained from the subjects after explanation of the treatment options and the risks and benefits of each procedure.

II. Methods

All patients were subjected to the following:

1. Detailed History taking including

- Age
- Gender.
- Associated renal problems.
- Visual complaints.
- Previous eye surgery.
- Duration of diabetes

2. Pre operative evaluation thorough ophthalmologic examination and imaging:

- **VA testing** with and without correction using Snellen's chart (metric) For statistical analysis, Snellen VA was converted to decimal fraction of vision to record the number of lines gained or lost after injection.
  (Table II)

- **Anterior segment evaluation** was done especially, for corneal or lenticular opacities, presence of rubeosis iridis, and intraocular pressure measurement by applanation tonometry.

- **Fundus examination** by contact and non contact slit-lamp biomicroscopy.

Table (II): Different notation of visual acuity values as decimal values, Snellen fractions [122]

Decimal	Snellen Fractions
0.016	1/60
0.033	2/60
0.05	3/60
0.066	4/60
0.083	5/60
0.10	6/60
0.12	6/48
0.16	6/38
0.20	6/30
0.25	6/24
0.32	6/19
0.40	6/15
0.50	6/12
0.63	6/9.5
0.80	6/7.5
1.00	6/6.0
1.25	6/4.8
1.63	6/3.8
2.00	6/3.0
2.50	6/2.4

Imaging:

- **Colored fundus photography and fluorescein angiography:**

Fundus photography on center of macula & Fluorescein angiography will be performed after intravenous injection of 5 ml 10% sodium fluorescein.

- **Optical coherance tomography (OCT):**

Oct scanning using Cirrus™ SD-OCT (Carl Zeiss Meditec, Inc. Dublin, CA) will be performed and quantify photoreceptor outer segment (PROS) length one week before injection of triamcinolone acetonide injection and one month after injection then after 6 months.

Consecutive patients with DME who were seen at the Ophthalmology Outpatient Clinic at Alexandria Main University Hospital. Over a two-month period were enrolled in this prospective study.

Best-corrected visual acuity was measured using ETDRS charts. Eyes with significant media opacities which can result in poor OCT signal were excluded. Eyes with other conditions that can cause macular thickening such as venous occlusion, epiretinal membrane, and/or vitreomacular traction were also excluded.

All individual B-scans from each OCT session were manually inspected to ensure proper delineation of the internal limiting membrane (ILM) and retinal pigment epithelium (RPE).

Eyes with subretinal fluid were not included, nor were scans with significant hard exudates or intraretinal fluid that caused improper delineation of the ILM/ RPE by the™ SD-OCT (Carl Zeiss Meditec,Inc. Dublin, CA). In the presence of subretinal fluid, the distance segment length. A correct measurement would require subtracting the thickness of the fluid pocket, which is not possible without a separate subretinal fluid segmentation algorithm.

Hard exudates and intraretinal fluid can obscure the underlying retina with their shadows. If the shadowed area is small enough, an RPE or IS/OS algorithm can reasonably interpolate across it, but for a very large shadowed regions it is unwise to do so. Only one eye in each patient was studied per study visit; if both eyes of a patient were eligible, the study eye was randomly selected and the fellow eye was allowed to be enrolled on an alternate study visit.

The study was performed with informed patient consent.

Optical Coherence Tomography—Scanning with the™ SD-OCT (Carl Zeiss Meditec, Inc. Dublin, CA). was performed using the 512 × 128 scan pattern where a 6mm × 6mm macular grid was scanned with 128 horizontal B-scan lines, each consisting of 512 A-scans per line (total of 65,536 sampled points). Each study eye was pharmacologically dilated prior to OCT scanning. All scans were performed by the same certified OCT technician. A total of three "high-quality" scans were obtained; these were defined as scans with a signal strength ≥6 that exhibit correct delineation of the ILM and RPE as detected automatically by the intrinsic software segmentation algorithm. The macular grid was

centered on the intrinsic fixation target during OCT scanning, and decentration of the grid by the technician in order to attempt to center the grid on the fovea was not allowed. Hence, the center of the macular grid was maintained at the patients' point of fixation.

Determination of Macular Thickness and Photoreceptor Outer Segment

Length— ™ SD-OCT (Carl Zeiss Meditec,Inc. Dublin, CA)data was processed for macular thickness and PROS length measurements without post-processing image alignment. These measurements were obtained for three macular parameters: macular grid (6mm × 6mm), the central subfield (1mm diameter), as well as for the central foveal point (0.33mm diameter). Macular thickness measurements were derived from the software (Cirrus™ 3.0, Carl Zeiss Meditec, Inc., Dublin, CA) provided by the manufacturer.

In order to calculate PROS length, The PROS was identified as the region between the RPE segmentation provided in (Cirrus™ 3.0, Carl Zeiss Meditec, Inc., Dublin, CA) and a prototype segmentation of the IS/OS boundary on each individual B-scan, and IS/OS segmentations were performed for all the B-scans on each macular grid.

The prototype IS/OS segmentation software filtered the image data to reduce speckle and identified consistent bands near the RPE where the image transitioned from dark to bright. Once the interior edge of the IS/OS was thus located, the point of maximum brightness just below this edge was identified as the preliminary location of the IS/OS. Lateral smoothing was then applied to give the final result.

Manual manipulation of OCT data was performed in the calculation of PROS Length. Exclusion of OCT scans was done based on the B-scans obtained from the (Cirrus™ 3.0, Carl Zeiss Meditec, Inc., Dublin, CA) software.

3. Procedure of intravitral injection

Topical anesthesia by Benoxinate HCL 0.4% eye drops.

Technique

The intravitreal injection of triamcinolone acetonide (trade name: Kenalog®,Bristol – Myers Squibb Company, New York, Cairo) will performed at same day.

1- Prior to the intravitreal triamcinolone acetonide injection, topical betadine (povidine iodine 5%) will be applied, and washed out after 2-3 minutes, then The patient will be completely draped.

2- Lid speculum will be inserted and paracentesis carried out to decrease the Intraocular pressure of the eye.

3- Injection of 4 mg /0.1 ml triamcinolone acetonide will be performed through 27-Gauge needle through the inferior parsplana, at 4 mm from the limbus.

4- Antibiotic eye drops will be applied.

4. Post operative evaluation

1- The patients were evaluated 1 day after intravitreal triamcinolone acetonide injection for the intraocoular pressure, inflammation and BCVA.

2- Then the patient were evaluated after one month for BCVA, anterior Segment, intraocular pressure, fundus, and photoreceptor outer segment (PROS) length by OCT. Visual acuity will be determined by observer performing best corrected refractmetry, and using snellen charts.

3- Then patient were evaluated after 6 months BCVA, anterior segment, intraocular pressure, fundus, and photoreceptor outer segment (PROS) length by OCT.

RESULTS

Demographic data:

This study was carried on 30 eyes of 22diabetic patients suffering from diabetic macular edema the cases were chosen according to the criteria mentioned before. Thirteen patients were males (60%) and nine were females (40%). Their ages were ranged from 39 to 70 years.

Table (III): Distribution of the studied cases according to demographic data

	No.	%
Sex		
Male	13	60.0
Female	9	40.0
Age		
≤50	10	33.3
51 – 60	9	30.0
61 – 70	11	36.7
Min. – Max.	39.0 – 70.0	
Mean ± SD	56.60 ± 8.43	
Median	58.0	

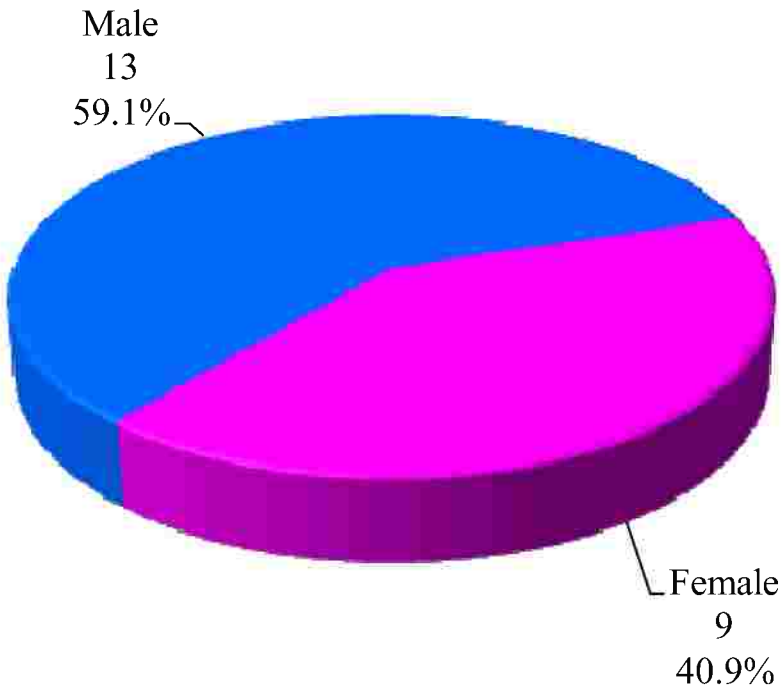


Figure (15): Distribution of the studied cases according to sex

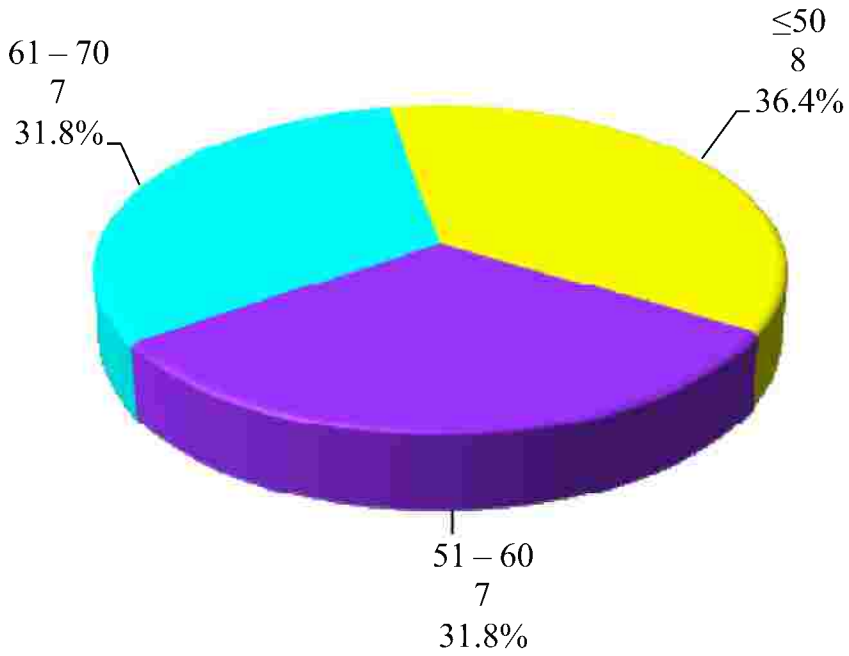


Figure (16): Distribution of the studied cases according to age

Types and duration of diabetes mellitus:

7 patients has type 1 diabetes(30%), 15 patients has type 2 diabetes(70%) and the duration of diabetes mellitus ranged from 6 years to 36 years, table (IV)

Table (IV): Distribution of the studied cases according to DM

	No.	%
Type of DM		
1	7	30.0
2	15	70.0
Duration of DM		
Min. – Max.	6.0 – 36.0	
Mean ± SD	16.67 ± 7.61	
Median	15.0	

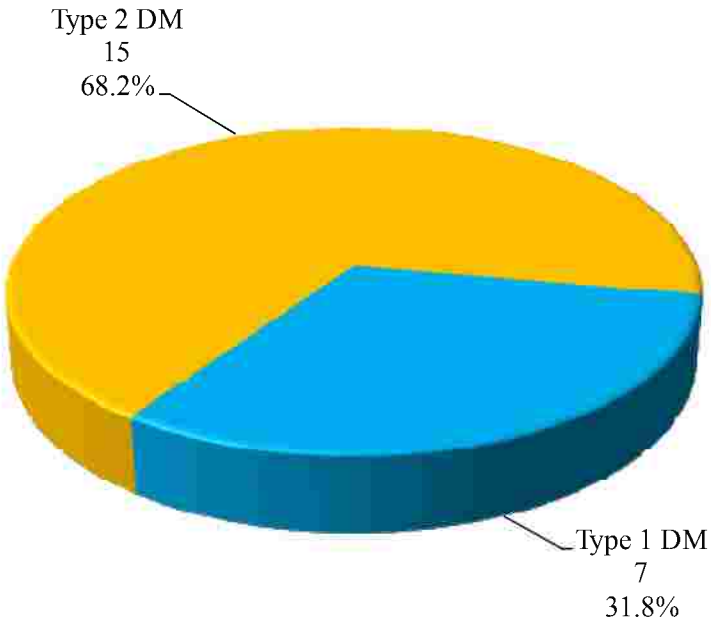


Figure (17): Distribution of the studied cases according to DM

Visual acuity:

This readings show BCVA Before IVTA , which was ranged from (4\60) snellen (0.07) decimal to 6\18 snellen 0.5 decimal with mean ±SD 0.16±0.08.

One month after IVTA injection, The BCVA was ranged from (6\36) snellen (0.10) decimal to (6\9) snellen (0.63) decimal ($p<0.001$) with mean of 0.27±0.16 . one eye gained three lines , 7 eyes gained 2 lines , 16 eyes gained one line and 6 eyes did not loss or gain any new lines .

After 6 monthes from IVTA injection , The BCVA was ranged from (5\60) snellen 0.08 decimal to (6\9) snellen (0.63) decimal ($p<0.001$) with mean 0.24±0.13. 4 eyes gained one line , 12 eyes did not loss or gain any new lines , 13 eyes lost one line and one eye lost 2 lines , due to reincreasing of photoreceptor thickness and development of cataract in 3 eyes. Table (V)

Comparison to the preoperative value:

- There was statistically significant improving in BCVA in most of cases one and six months after IVTA injection.

Table (V) Mean, standard deviation and range of visual acuity before IVTA injection and after one month and six months from IVTA injection.

	Before injection	After		p
		1 month from injection	6 months from injection	
VA				
Min. – Max.	0.07 – 0.32	0.10 – 0.63	0.08 – 0.63	
Mean ± SD	0.16±0.08	0.27±0.16	0.24±0.13	<0.001*
Median	0.13	0.25	0.25	
$^{WRST}p_1$		<0.001*	<0.001*	
$^{WRST}p_2$		0.092		
Mean % of change		↑77.33	↑65.30	

p: p value for Friedman test
WSRT: Wilcoxon signed ranks test
p_1: p value for comparing between before and after injection
p_2: p value for comparing between 1 month from injection with other stages
*: Statistically significant at $p \leq 0.05$

Figure (18): Mean, standard deviation and range of visual acuity before IVTA injection and after one month and six months from IVTA injection.

OCT changes:

1- Retinal thickness:

These readings show the retinal thickness of central subfield zone at the base line evaluation, one month and 6 months after IVTA injection. Before IVTA injection the retinal thickness was ranged from 369.0 – 543.0 μm with mean of 432.37±47.99 μm.

One month after IVTA injection the thickness was ranged from 237.0 – 400.0 μm. With mean of 313.43±48.04 μm.

After 6 months from IVTA injection the thickness was ranged from 245.0 – 440.0 μm. With mean of 325.80±53.87 μm. Table (VI)

Comparison to the preoperative value:

- There was statistically significant changes in the retinal thickness one and six months after IVTA. As after one month it was observed to be decreased and after 6 months revealed increasing but still lower than that measured before IVTA injection.

Table (VI): Mean standard deviation and range of retinal thickness in central subfield zone before IVTA injection and after one month and six months from IVTA injection.

	Before injection	After		p
		1 month from injection	6 months from injection	
Retinal thickness				
Min. – Max.	369.0 – 543.0	237.0 – 400.0	245.0 – 440.0	
Mean ± SD	432.37±47.99	313.43±48.04	325.80±53.87	<0.001*
Median	434.50	307.0	310.50	
$^{Bon}p_1$		<0.001*	<0.001*	
$^{Bon}p_2$		<0.001*		
Mean % of change		↓27.25	↓24.39	

p:p value for F test (ANOVA) with repeated measures
Bon: Stands for adjusted Bonferroni p-value for ANOVA with repeated measures
p_1: p value for comparison between before and after injection
p_2: p value for comparison between 1 month and 6 months after injection
*: Statistically significant at $p \leq 0.05$

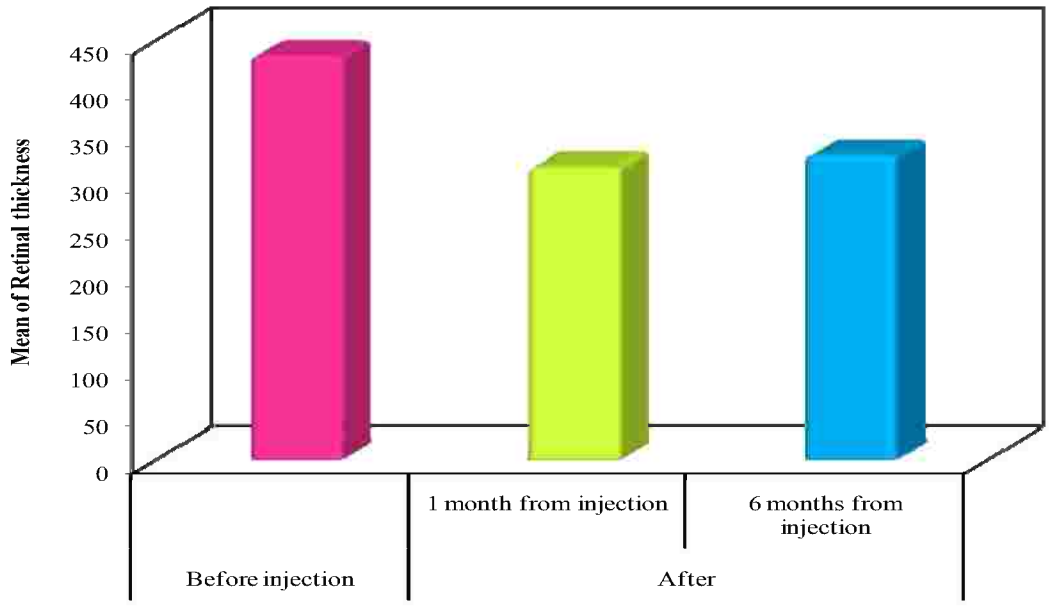


Figure (19): Mean, standard deviation and range of retinal thickness in central subfield zone before IVTA injection and after one month and six months from IVTA injection

2- Photo receptor outer segment lengh changes:

A) Macular grid area:

These readings show the Photo receptor outer segment length before IVTA in the macular grid (6mm x6mm) which was ranged from15.0µm – 40.0µm with mean±SD 29.60±7.00 µm

One month after IVTA the PROS length was ranged, 13.0µm – 35.0µm with mean±SD 23.80±6.03 µm ($p<0.001$)

Six months after IVTA, the PROS length was ranged from 14.0µ–35.0µ with mean 25.67±6.25 ($p<0.001$). Table (VII)

Comparison to the preoperative value:

- There was statistically significant decrease PROS length in macular grid zone one and six months after IVTA

Table (VII): Mean, standard deviation and range of PROS length in macular grid zone before IVTA injection and after one month and six months from IVTA injection.

	Before injection	After		p
		1 month from injection	6 months from injection	
MG				
Min. – Max.	15.0 – 40.0	13.0 – 35.0	14.0 – 35.0	
Mean ± SD	29.60±7.00	23.80±6.03	25.67±6.25	<0.001*
Median	30.0	24.50	25.50	
$^{Bon}p_1$		<0.001*	<0.001*	
$^{Bon}p_2$		<0.001*		
Mean % of change		↓19.65	↓13.04	

p:p value for F test (ANOVA) with repeated measures
Bon: Stands for adjusted Bonferroni p-value for ANOVA with repeated measures
p_1: p value for comparison between before and after injection
p_2: p value for comparison between 1 month and 6 months after injection
*: Statistically significant at $p \leq 0.05$

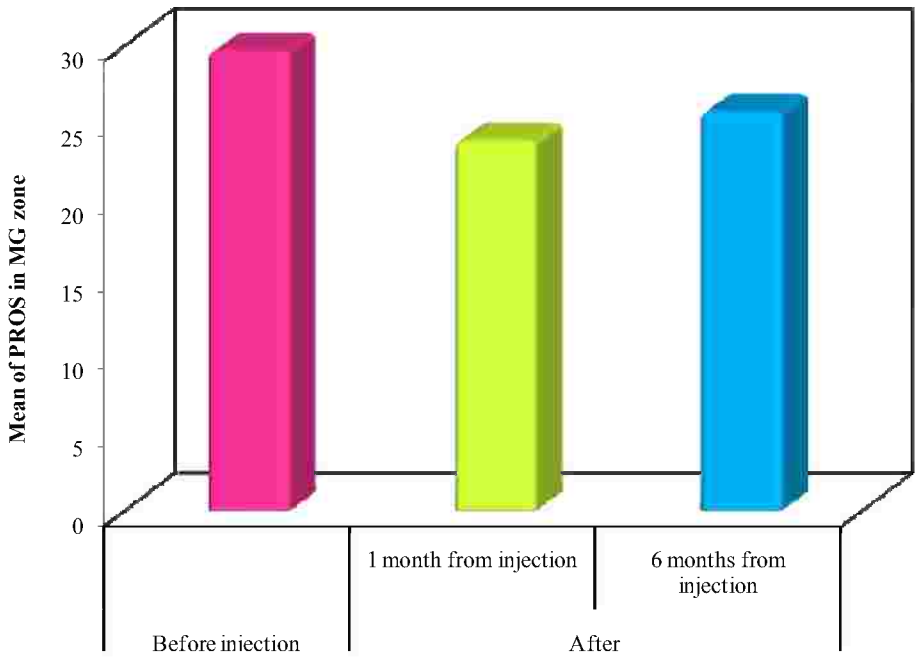


Figure (20): Mean, standard deviation and range of PROS length in macular grid zone before IVTA injection and after one month and six months from IVTA injection

B) Central subfield zone:

These readings show Photo receptor outer segment length before IVTA in the central subfield (I mm diameter) which was ranged from 15.0 -41.0µm with mean 29.33±7.00 µm, one month after IVTA the PROS length ranged from 12.0 -34.0 µm with mean ±SD 23.90±6.02 µm($p<0.001$).

6 months after IVTA PROS length was ranged from 14.0-36.0 µm with mean ±SD 25.57±6.35 µm ($p<0.001$) , Table (VIII)

Comparison to the preoperative value:

- There was statistically significant decrease in PROS lengh in central subfield zone one and six months after IVTA.

Table (VIII): Mean, standard deviation and range of PROS length in central subfield zone before IVTA injection and after one month and six months from IVTA injection.

	Before injection	After		p
		1 month from injection	6 months from injection	
CS				
Min. – Max.	15.0 – 41.0	12.0 – 34.0	14.0 – 36.0	
Mean ± SD	29.33±7.00	23.90±6.02	25.57±6.35	<0.001*
Median	30.0	24.0	25.50	
$^{Bon}p_1$		<0.001*	<0.001*	
$^{Bon}p_2$		<0.001*		
Mean % of change		↓18.55	↓12.56	

p:p value for F test (ANOVA) with repeated measures
Bon: Stands for adjusted Bonferroni p-value for ANOVA with repeated measures
p_1: p value for comparison between before and after injection
p_2: p value for comparison between 1 month and 6 months after injection
*: Statistically significant at $p \leq 0.05$

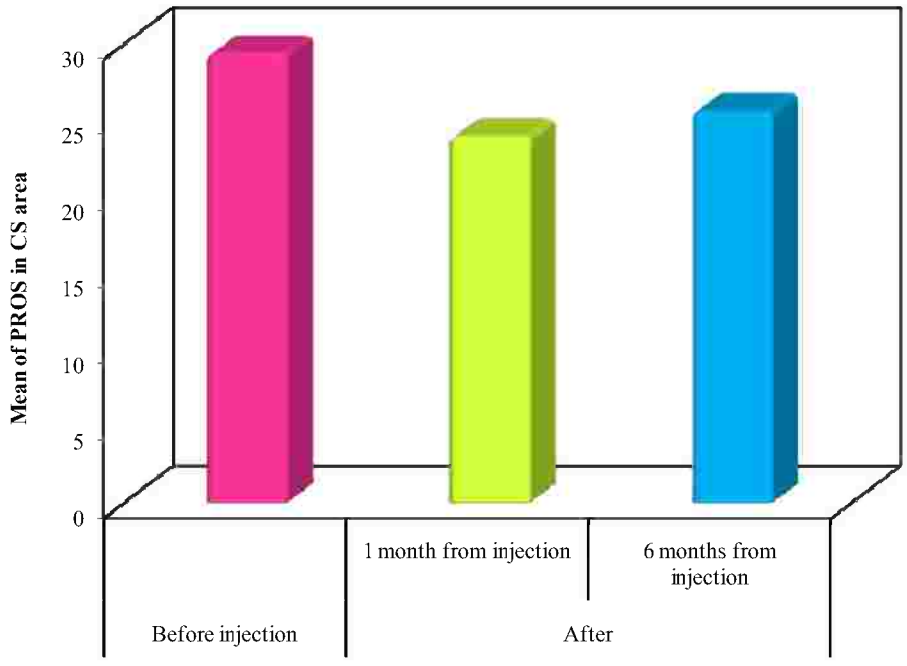


Figure (21): Mean, standard deviation and range of PROS length in central subfield zone before IVTA injection and after one month and six months from IVTA injection

C) Central foveal point

These reading show photo receptor outer segment length before IVTA injection in the central fovial point (0.33mm diameter) which was ranged from 14-40 µm with mean ±SD 29.87±7.33 µm .

One month after IVTA the PROS length was ranged from 11.0 – 33.0 µm with mean ±SD 24.13±6.21 µm($p<0.001$).

6 months after IVTA PROS length was ranged from14.0 – 35.0 µm with mean ±SD 25.97±6.50 ($p<0.001$) Table (IX)

Table (IX): Mean, standard deviation and range of PROS length in central foveal point before IVTA injection and after one month and six months from IVTA injection.

	Before injection	After		p
		1 month from injection	6 months from injection	
CFP				
Min. – Max.	14.0 – 40.0	11.0 – 33.0	14.0 – 35.0	
Mean ± SD	29.87±7.33	24.13±6.21	25.97±6.50	<0.001*
Median	30.0	24.0	26.0	
$^{Bon}p_1$		<0.001*	<0.001*	
$^{Bon}p_2$		<0.001*		
Mean % of change		↓19.25	↓12.58	

p:p value for F test (ANOVA) with repeated measures
Bon: Stands for adjusted Bonferroni p-value for ANOVA with repeated measures
p_1: p value for comparison between before and after injection
p_2: p value for comparison between 1 month and 6 months after injection
*: Statistically significant at $p \leq 0.05$

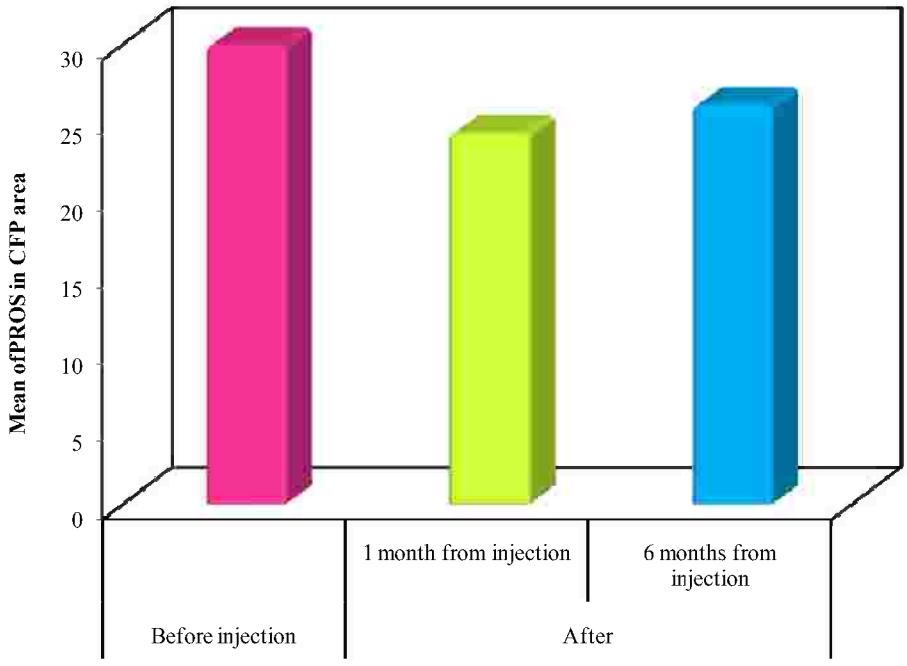


Figure (22): Mean, standard deviation and range of PROS length in central foveal point before IVTA injection and after one month and six months from IVTA injection

Correlation between PROS length, retinal thickness and visual acuity in central subfield zone:

Correlation coefficients were performed In order to quantitatively assess the relationship between retinal thickness, PROS and visual acuity. The slope of PROS length vs. visual acuity was more statistically significant than the macular thickness vs. visual acuity .**Tables (X, XI).**

Table (X): Correlation between VA with retinal thickness in the central subfield zone

	VA					
	Before injection		1 month from injection		6 months from injection	
	r_s	p	r_s	p	r_s	p
Retinal thickness	-0.625*	<0.001	-0.734*	<0.001	-0.649*	<0.001

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

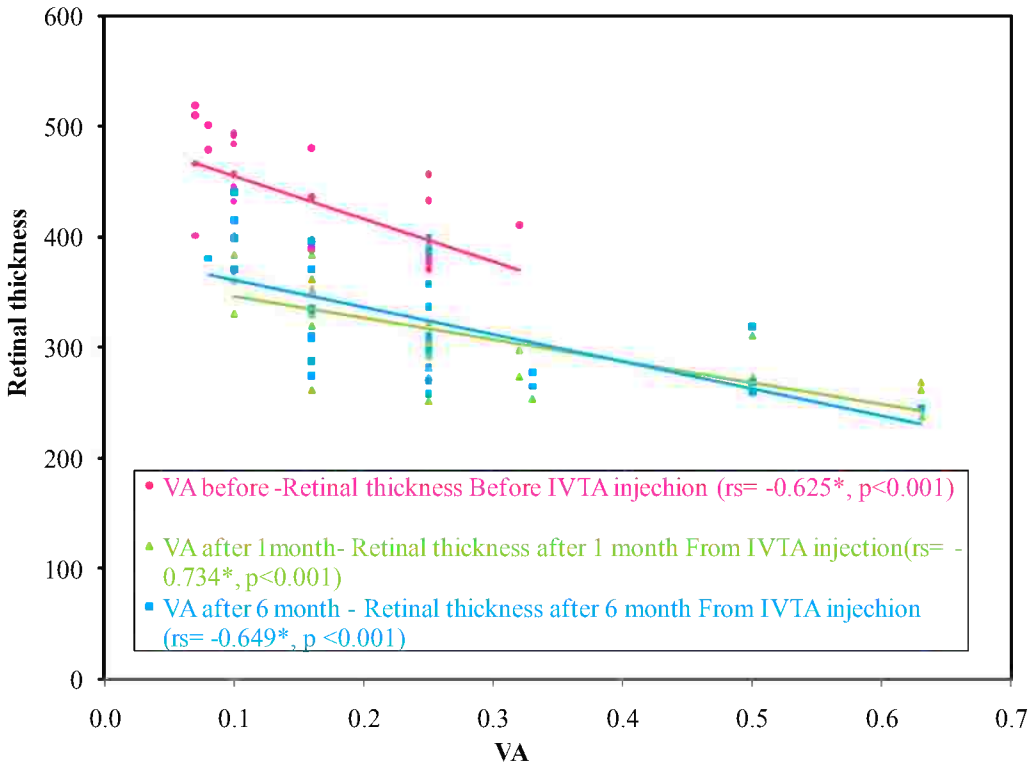


Figure (23): Correlation between VA with retinal thickness in the central subfield zone

Table (XI): Correlation between VA and PROS in MG, CS and CFP

	VA					
	Before injection		1 month from injection		6 months from injection	
	r_s	p	r_s	p	r_s	p
MG	-0.893*	<0.001	-0.836*	<0.001	-0.916*	<0.001
CS	-0.892*	<0.001	-0.812*	<0.001	-0.910*	<0.001
CFP	-0.875*	<0.001	-0.797*	<0.001	-0.942*	<0.001

r_s: Spearman coefficient
*: Statistically significant at $p \leq 0.05$

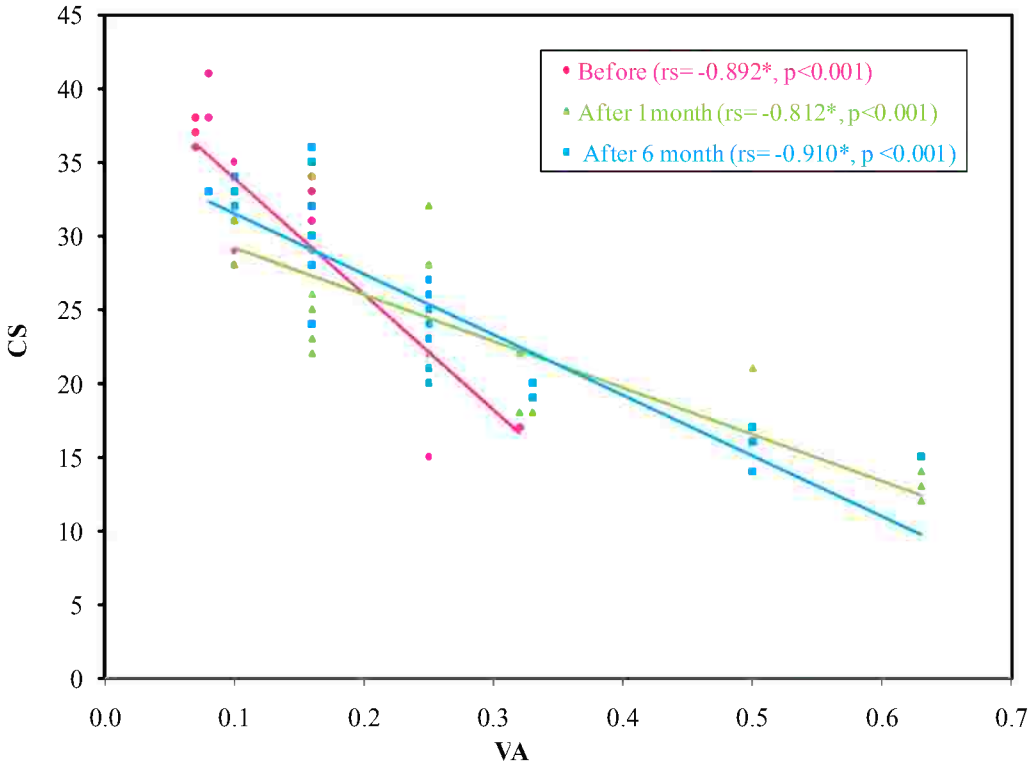


Figure (24): Correlation between VA and PROS in CS

Case 1

Male patient 69 years old type 2 DM Duration of 18 years.

Pre injection:

- BCVA is 4/60 _0.066 Retinal thickness 543 µm PROS IN CS zone IS 39 µm.

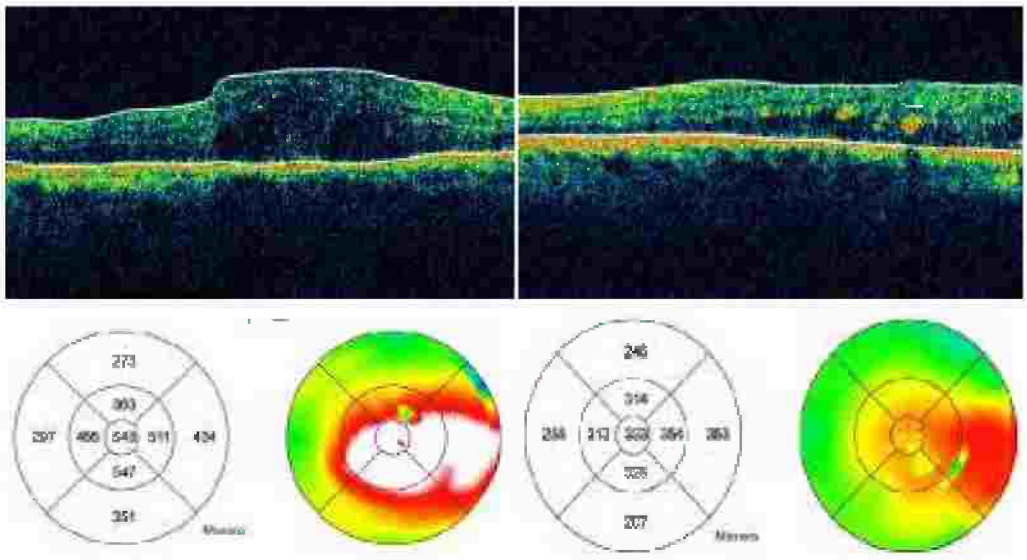
1 month post injection

- BCVA is 6/36 _ 0.16. Retinal thickness 333 µm. PROS IN CS zone IS 31 µm.

6 months post injection

- BCVA is 6/60 _ 0.10. Retinal thickness 366 µm. PROS IN CS zone IS 33 µm

pre injection 1 month post injection



6 months post injection

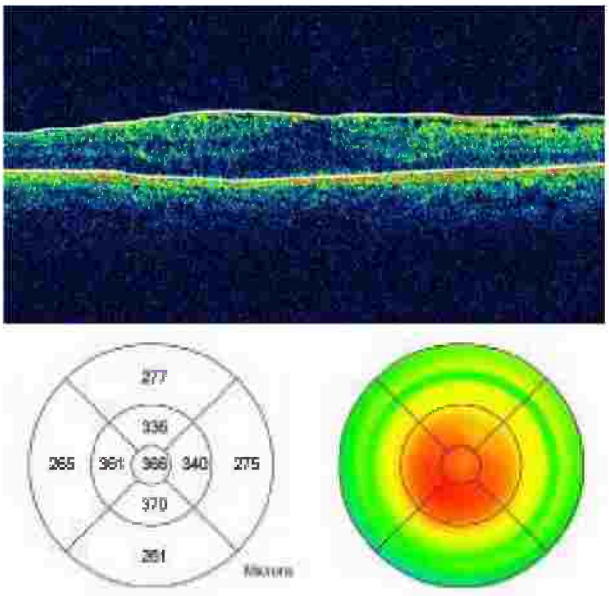


Figure (25): Case 1. OCT scan (pre-IVTA injection, 1 and 6months after IVTA injection). Macular thickness maps both numerical and colour (pre-IVTA injection, 1 and 6 months after IVTA injection) showing the central area thickness which decreased from 543 µm to 333 µm and increased to 366 µm.

Case 2

Female patient 37 years old. Type 1 DM. Duration of DM 21 years.

Pre injection:

- BCVA is 6/24 _0.25. Retinal thickness 366 µm. PROS IN CS zone IS 21 µm.

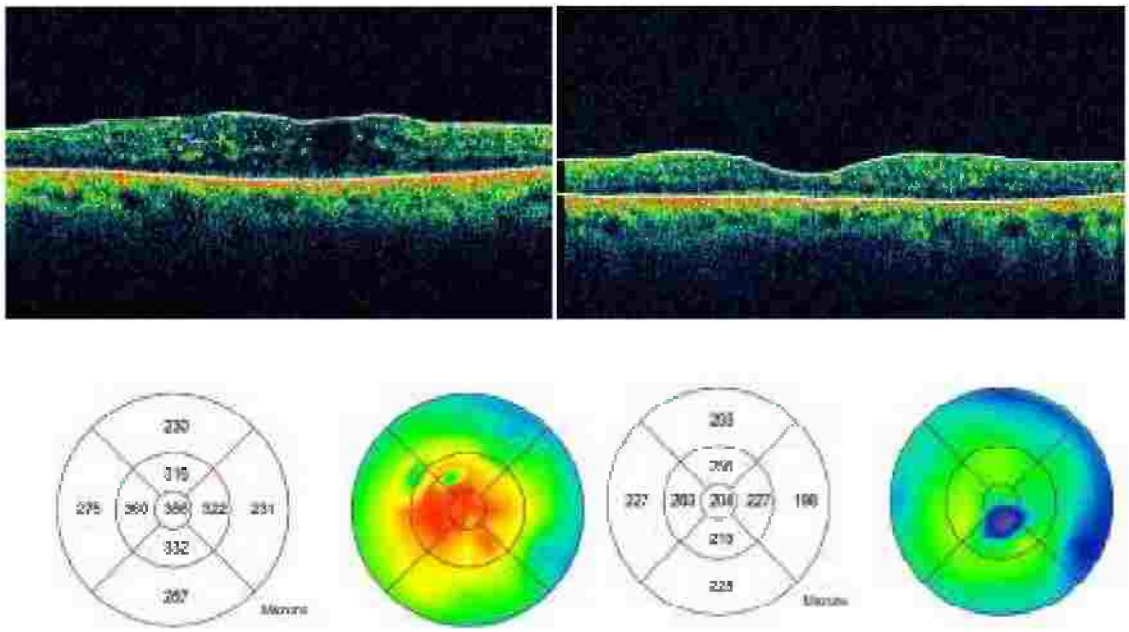
1 month post injection

- BCVA is 6/18 _0.32. Retinal thickness 204 µm. PROS IN CS zone IS 18 µm.

6 months post injection

- BCVA is 6/18 _0.25. Retinal thickness 225 µm. PROS INCS zone IS 20 µm

pre injection 1 month post injection



6 months post injection

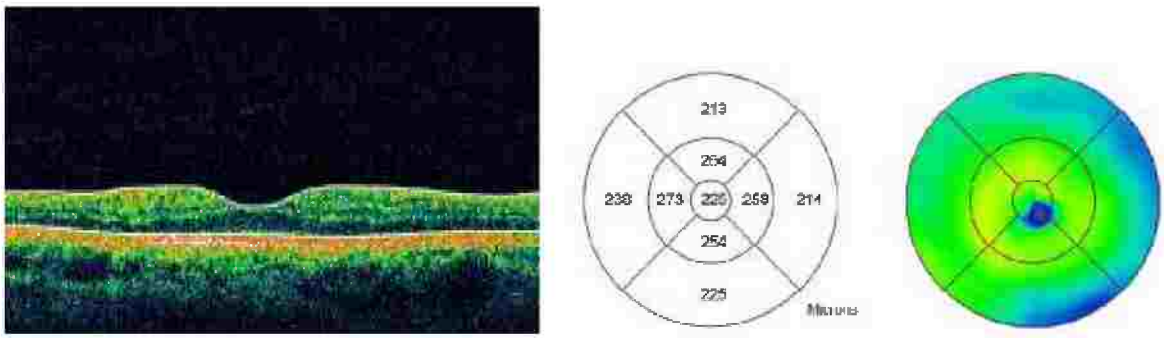


Figure (26): **Case 2.** OCT scans (pre-IVTA injection, 1 and 6 months after IVTA injection). Macular thickness maps both numerical and colour (pre-IVTA injection, 1 and 6 months after IVTA injection) showing the central area thickness which decreased from 366 µm to 204 µm and increased to 225 µm