Research Article

Functional and anatomical treatment effect after intravitreal injection of ranibizumab for diabetic macular edema

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Abstract
Purpose: To evaluate treatment effect of intravitreal injection of ranibizumab for diabetic macular edema (DME) both functionally by changes of best corrected visual acuity (BCVA) and anatomically by changes of optical coherence tomography (OCT) central subfield thickness. **Methods** Twenty eyes of fifteen patients with diffuse DME were treated with intravitreal injection of ranibizumab. BCVA measured by Snellen acuity chart and central subfield thickness measured by OCT before and ٣ months after intravitreal injection of ranibizumab. The pretreatment values of BCVA and OCT central subfield thickness were compared with the corresponding values at ٣ months after intravitreal injection of ranibizumab. **Results:** At ٣ months, the BCVA was significantly improved (P<٠.٠٠١) and the central subfield thickness was significantly reduced (P<٠.٠٠١). **Conclusions:** intravitreal injection of ranibizumab for DME significantly improve BCVA and reduce OCT central subfield thickness ٣ months after treatment
Keywords: diabetic macular edema; optical coherence tomography; intravitreal injection of ranibizumab

Introduction
DME is the most important cause of visual deterioration in working-age diabetic patients.[١] The previous treatment standard for DME was laser photocoagulation but there are different degrees of complications. Currently, intra-vitreal injection of anti VEGF with or without laser photocoagulation is the standard of care for patients with DME.[٢] a quantity of randomized multicenter studies had proven that repeated intravitreal injections of anti-VEGF have superior outcomes in patients with DME when compared to laser treatment alone.[٣] Nguyen et al. proposed that intravitreal injection of anti-VEGF should be the first-line of treatment of DME.[٤]

The purpose of this study to evaluate treatment effect of intravitreal injection of ranibizumab for DME both functionally by changes of BCVA and anatomically by changes of OCT central subfield thickness.

Patients and methods
This study was an interventional prospective comparative nonrandomized study performed in the Ophthalmology Department of Minia University Hospital between November ٢٠١٥ to January ٢٠١٧. The study was approved by the Ethics Committee of Minia University. The aim of the study and the used methodology were thoroughly explained to the patients and informed consent was obtained.
Twenty eyes of fifteen patients (٥ males and ١٠ females) were included in the study.

Inclusion criteria:
- Type ٢ DM patients with centrally involving DME

Exclusion criteria:
- Patients with type ١ DM
- History of cerebral stroke or myocardial infarction
- Patients with macular ischemia detected by fluorescein angiography
- Patients with vitreoretinal traction and interruption of external limiting membrane (ELM) or inner segment-outer segment (IS-OS) junction detected by OCT
- Patients with proliferative retinopathy

- Previous focal or grid conventional or SMD laser photocoagulation
- Previous intravitreal injection of steroid or anti-VEGF
- Previous vitreo retinal surgery
- Previous cataract surgery within six months
- Patients with media opacities interfering with adequate fundus observation
- Patients with macular diseases other than DME such as age-related macular degeneration (AMD)
- Patients with optic disc pathology or other ocular disorders that may affect visual outcome
- Patient with lack of regular follow-up

At baseline, all patients were subjected to a detailed assessment including the following:

١) Complete history taking including:

- Duration of DM
- Past glycemic control (HbA١c)
- Medications
- General medical history (e.g., renal disease, systemic hypertension, serum lipid levels and pregnancy)
- Ocular history (e.g., trauma, other eye diseases, ocular injections, photocoagulation and ocular surgery)

٢) Full ophthalmological examination including:

- BCVA using Snellen VA chart then converted to decimal acuity for statistical analysis.
- Pupillary assessment
- Anterior segment examination using Slit-lamp biomicroscopy
- Detailed fundus examination with indirect ophthalmoscopy and stereoscopic examination using +٧٨D lens to assess the DME and other diabetic changes in the fundus
- Intraocular pressure (IOP) measurement using applanation tonometry

٣) Ophthalmological investigations:

A) Colored fundus photography and fluorescein angiography:

This was performed using (**Topcon)™** fundus camera to detect site of leakage and macular ischemia. ٥ ml of ١٠٪ sodium fluorescein solution dye is injected intravenously through an antecubital vein. Images are acquired immediately after injection and continue for ten minutes.

B**) OCT examination**:

Examinations were done using the Cirrus HD-OCT ٤٠٠٠ **(Zeiss, Germany)™**. After pupillary dilatation two scan types were done for each eye. The first is the macular cube ٥١٢ × ١٢٨. This generates a cube of data through a ٦ mm^2 grid by acquiring a series of ١٢٨ horizontal scan lines each composed of ٥١٢ A-scans, except for the central high-definition vertical and horizontal scans, which are composed of ١,٠٢٤ A-scans; this scan helps in giving an idea about the regions of maximum height so they are better assessed and scanned by the high-definition ٥-line raster. The second scan is the high-definition five-line raster. This scan gives the highest resolution of all the Cirrus scan types. It scans through five parallel lines of equal length, and each line is composed of ٤,٠٩٦ A-scans. It is used to evaluate the mean retinal thickness from the innermost ILM to the RPE at the fovea. Multiple scans were taken throughout the whole macular area. Scans with the strongest signal strength were selected (signal strength of more than ٦/١٠).

In this study the central subfield retinal thickness in the macular cube map, which is the central ١-mm diameter circular zone representing the foveal area was used for statistical analysis before and after treatment of DME by intravitreal injection of ranibizumab or SDM. Any disruption of the ELM was searched for within the central ١ mm of the fovea. If the ELM line appeared to be complete at the fovea in all scans, this was considered as an intact ELM. Any discontinuity or interruption of the ELM line in one scan or more was considered a disrupted ELM layer. The integrity of the IS/OS line beneath the fovea was evaluated using the same criteria described for the ELM line. If the line appeared to be complete at the fovea in all scans, it was diagnosed as an intact IS/OS line.

If there was an incomplete IS/OS line in one scan or more, it was considered a

disrupted IS/OS layer. Other findings that were evaluated during scan analysis included neurosensory detachment and vitreoretinal traction.

Ranibizumab (RNBZ) was injected at doses of ٠.٥ mg in ٠.٠٥ ml, according to the pro re nata (PRN) protocol with injections at baseline and at ١-month and ٢-months, a total of three injections as a loading dose administered to all patients. Next, we evaluated the patients and determined the follow-up injections according to the presence of fluid in the macula (more than ٣٠٠um central subfield thickness in OCT) and/or BCVA was reduced by ٥ letters.

Treatment technique

Intravitreal injections were done in the operating theater under complete aseptic conditions as follow:

- The pupil was dilated using tropicamide ١٪.
- Topical anesthesia with Benoxinate HCL ٠.٤ % eye drops.
- Ocular surface sterilization with betadine eye drops ٥٪ and sterile drapes were applied.
- Insertion of an eye lid speculum, ٠.٥ mg (٠.٠٥ mL) ranibizumab was injected through pars plana with a ٢٨-gauge needle through the inferior temporal quadrant ٤ mm behind the limbus in phakic patients and ٣,٥ mm behind the limbus in pseudophakic patients
- Central retinal artery perfusion and intraocular pressure (IOP) were assessed just after injection
- Paracentesis was done if IOP was markedly increased.
- Application of eye drops (Topical antibiotic moxifloxacin ٥mg /ml was prescribed for ٥ days
- Eye patching by sterile dressing
- Follow up Day ١, day ٣, day ٥ and ١ week after the injection to detect any complication

Follow-up examination was performed ٣ months after treatment with recording of the BCVA and central subfield thickness on OCT. These recorded data then compared with base line data

Statistical analysis

Data were analyzed using the statistical package for the social sciences (SPSS, version ٢٠) software.one way ANOVA test was used for parametric quantitative data between the ٣ groups. Independent sample t test was used for parametric quantitative data between ٢ groups. Paired sample t test was used for parametric quantitative data within each group. The level of significance P value significant if < ٠.٠٥.

Results

٢٠ eyes of ١٥ patients with a mean age of ٦١.٥ ± ٣.٣ years and mean duration of DM ١٣.٩ ± ٣.٨ years were enrolled in this study. None of the eyes were excluded from the study during follow-up. Demographic and baseline characteristics of the patients are illustrated in table ١.

Table١: Demographic and general characteristics of the patients

	(n=١٥)
Age Range Mean ± SD	 (٥٢-٦٥) ٦١.٥±٣.٣
DM duration Range Mean ± SD	 (٧-٢٠) ١٣.٩±٣.٨
Sex Male Female	 ٥(٣٣.٣٪) ١٠(٦٦.٧٪)
Side Unilateral Bilateral	 ١٠(٦٦.٧٪) ٥(٣٣.٣٪)

The mean and the standard deviation of BCVA (in decimal) before and ٣ months after treatment summarized in table ٢ There was significant improvement in BCVA before treatment and ٣ months with P value <٠.٠٠١.Also the mean changes of BCVA improvement was ٩٧.٦±٤٦.٦%

Table ٢: illustrate baseline BCVA and ٣ months after treatment.

	(n=٢٠)
(١)BCVA pre Range Mean ± SD	 (٠.٠٦-٠.٢٥) ٠.١٤±٠.٠٧
(٢)BCVA post Range Mean ± SD	 (٠.١-٠.٥) ٠.٢٦±٠.٠٩
(٣)% increase BCVA Mean ± SD	 ٩٧.٦±٤٦.٦
(٤)P value **(pre vs post)**	 **<٠.٠٠١***

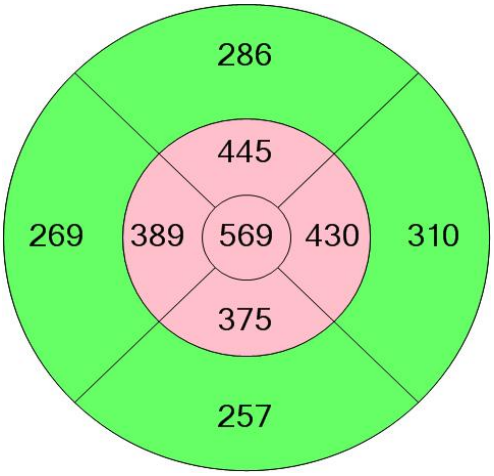
*: Significant level at P value < ٠.٠٥

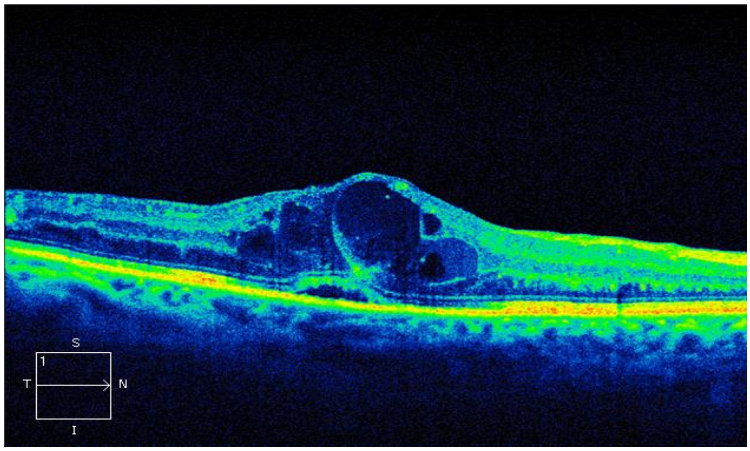
The mean and the standard deviation of central subfield thickness of OCT before and ٣ months after treatment summarized in table ٣. There was significant improvement in central subfield thickness before treatment and ٣ months after treatment with P value <٠.٠٠١.Also the mean changes of central subfield thickness improvement was ٣٣.٤±١٥.٦%.

Table ٣: illustrate OCT central subfield thickness at baseline and ٣ months after treatment.

	(n=٢٠)
(١)OCT pre Range Mean ± SD	 (٣١٤-٥٦٩) ٤١٧.٨±٨٥.٧
(١)OCT post Range Mean ± SD	 (١٩١-٣٨٠) ٢٧٠.١±٥٢.٤
(٢)% decrease OCT Mean ± SD	 ٣٣.٤±١٥.٦
(٣)P value (pre vs post)	 <٠.٠٠١*

*: Significant level at P value < ٠.٠٥





Case ١ baseline OCT revealed cystoid macular edema ,neurosnsory detachment and central subfield thickness ٥٦٩ um. treated by intravitreal injection of ranibizumab

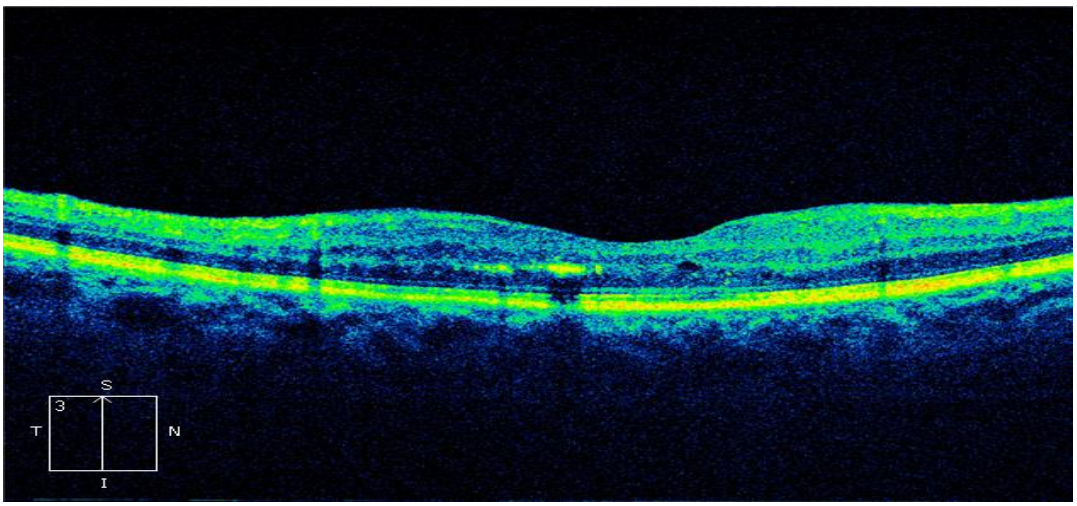


Figure ٢: Case ١ one month after third injection of ranibizumab OCT revealed resolution of cystoid macular edema and neurosnsory detachment and central subfield thickness reduced to ٢٤١ um.

Discussion

A quantity of randomized multicenter studies had proven that repeated intravitreal injections of anti-VEGF have superior outcomes in patients with DME when compared to laser treatment alone[٥,٣]. Nguyen et al. proposed that intravitreal injection of anti-VEGF should be the first-line of treatment of DME.[٤] RCTs have been proved the long-term efficacy of PRN dosing of ranibizumab in DME.[٦] All patients included in this group showed reduction in central subfield thickness below ٣٠٠um except two eyes needed further injection. All patients had no complications during the follow-up period and their fast blood glucose levels were stable. This study revealed that intravitreal injection of ranibizumab significantly improved BCVA from ٠,١٤±٠,٠٧ at base line to ٠,٢٦±٠,٠٩ after three months (P<٠,٠٠١) and the percent of increase was ٩٧.٦٪. Also OCT central subfield thickness significantly reduced from ٤١٧,٨± ٨٥.٧ um at base line to ٢٧٠,١±٥٢,٤um after three months (P<٠,٠٠١) and the percent of reduction was ٣٣,٤%

Significant improvement in BCVA due to significant reduction in macular edema. These findings are in accordance with previous studies evaluated anatomical and functional effect of treatment of DME by intravitreal injection of ranibizumab according to changes in BCVA and OCT central subfield thickness such as RESOLVE, RESTORE, RIDEand RISE studies

RESOLVE study was double-masked sham-controlled RCT evaluated ٠.٣ mg and ٠.٥ mg RBZ in three monthly doses for DME. Thereafter, the treatment could be stopped or re-initiated based on protocol defined criteria. More than ٦٠٪ of eyes treated with the RBZ had ≥١٠ letters gain compared to ١٨٪ of eyes with sham at ١ year (P < ٠.٠٠٠١).[٧] RESTORE study was RCTs conducted with an aim to demonstrate the superiority of ranibizumab over laser therapy. In the RESTORE study BCVA gain was highest in the RBZ monotherapy arm at the primary endpoint of month ١٢ (+٦.١ vs. +٠.٨ letters in laser arm; P < ٠.٠٠١). [٥] RIDE and RISE studies were multicenter RCTs that led to the approval of ranibizumab by the US FDA in ٢٠١٢. The studies compared two doses of monthly ranibizumab (٠.٣ mg and ٠.٥ mg) to sham injection in patients with DME. In both the studies, significantly higher number of patients treated with ranibizumab gained ≥١٥ letters at month ٢٤ compared to sham-treated group (٤٤.٨٪ vs. ١٨.١٪ in RISE; P < ٠.٠٠٠١, and ٣٣.٦٪ vs. ١٢.٣٪; P< ٠.٠٠٠١ in RIDE). [٤]

Conclusions

Intravitreal injection of ranibizumab for DME significantly improve BCVA and reduce OCT central subfield thickness ٣ months after treatment

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