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# Specular Microscopic Evaluation of Corneal Endothelium After Intravitreal Injection of Ranibizumab for Treatment of Diabetic Macular Edema

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## Abstract

**Background:** The eye disorder known as diabetes-related macular edema (DME) is frequent and dangerous. DME is one of the easiest conditions to treat, even though it can cause vision impairment. Photocoagulation, intravitreal medicines, and surgical excision are some of the therapy options currently available.

**Aim:** To evaluate how intravitreal ranibizumab injections affected corneal endothelial changes over 3 months in patients diagnosed with DME.

**Patients and methods:** A case–control study at Giza Memorial Institute of Ophthalmic Research from May 2022 to November 2022 compared patients with diabetes mellitus (DM) who have good glycemic control and patients who have poor glycemic control with macular edema. Ten eyes received 0.5 mg/0.05 ml ranibizumab intravitreal injections for three consecutive months. Specular microscopy for endothelial cell number and thickness of the cornea determination was done before administration and after this.

Three groups were divided into: group 1: 10 eyes of diabetic patients (type II) with macular edema and accompanying nonproliferative diabetic retinopathy and are going to undergo intravitreal injection of ranibizumab. Group 2: 10 eyes of diabetic patients without diabetic retinopathy. Group 3: 10 eyes of healthy adults of the same age group.

**Results:** Our research revealed that whereas endothelial cell hexagonality%, coefficient of variation, as well as central corneal thickness did not differ between DM patients and controls, DM patients proved to have lower endothelial cell density than healthy participants of identical sex and age.

**Conclusion:** Injection of anti-vascular endothelial growth factor intravitreally in DME patients showed no corneal endothelium or central corneal thickness changes after receiving ranibizumab 0.5 mg/0.05 ml. Therefore, the safety of intravitreal anti-vascular endothelial growth factor injections has been determined for the corneal endothelium. Extra researches are needed.

**Keywords:** Corneal endothelium, Diabetic macular odema, Anti-VEGF injection, Ranibizumab

## 1. Introduction

A common and dangerous eye condition is diabetes-related macular edema (DME). DME is among the most straightforward disorders to treat despite the fact that it has the potential to impair vision. Photocoagulation, intravitreal medication [either corticosteroid treatments or vascular

endothelial growth factor (VEGF) inhibitors], in addition to surgical excision, are some of the therapeutic options that are currently available. Laser therapy, which is for a long time, served as the gold standard, has been replaced by anti-VEGFs.<sup>1</sup>

A recombinant humanized monoclonal antibody called ranibizumab was created from the parent antibody bevacizumab.<sup>2</sup> VEGF gets attached by it,

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and this reduces VEGF signaling. Additionally, 3 days after ranibizumab was administered intravitreally to rabbit eyes, researchers discovered the antibody in the aqueous humor, reaching maximum level of 17.9 mg/ml.<sup>3</sup> So it makes sense to believe that ranibizumab impact the cornea's endothelial layer.

The human endothelial layer of the cornea, which faces the eye anterior chamber and lines the posterior aspect of the membrane of Descemet, is considered as nonregenerating. The corneal endothelium, which is metabolically active, pumps water out of the stroma into the aqueous humour to keep the stroma at its dehydrated condition of 70% of water. A specular microscope can examine the density in addition to the morphology of the endothelial layer of the cornea. It has been demonstrated that the specular microscope is trustworthy and reliable.<sup>4</sup>

Additionally, some researchers documented a few acute inflammation intraocularly following intravitreal ranibizumab injections. This raises some concerns about the safety of intravitreal ranibizumab injections. To determine ranibizumab's safety for the treatment of DME, this investigation's objective is to evaluate how intravitreal ranibizumab affects the corneal endothelium.

So, in our study, we aimed to evaluate how intravitreal ranibizumab injections affected corneal endothelial changes over 3 months in patients with DME.

## 2. Patients and methods

This study is a case–control study assessing ten consecutive patients who were treated for DME with 0.5 mg/0.05 ml intravitreal injection of ranibizumab for three consecutive months. Specular microscopy was performed on the central corneal thickness (CCT), coefficient of variation (CD), coefficient of variation (CV), hexagonality (HEX%). We compared the results between before and 30 days following the first, second, and third injections.

Three groups were divided into: group 1 included 10 eyes of diabetes mellitus (DM) cases (type 2) with ME and accompanying nonproliferative diabetic retinopathy who will undergo intravitreal injection of ranibizumab. Group 2 included 10 eyes of diabetic patients (type 2) without evidence of diabetic retinopathy. Group 3 included 10 eyes of healthy adults of the same age group.

### 2.1. Inclusion criteria

Patients in the age group (35–65 years), diabetes type 2, no previous ocular surgery, no previous ocular inflammation or trauma.

### 2.2. Exclusion criteria

History of previous ocular surgery, proliferative stage of diabetic retinopathy, diabetes type 1, glaucomatous patients, and any other systemic diseases affecting the eye.

This study was carried out in Giza Memorial Institute of Ophthalmic Research from May 2022 to November 2022 on diabetic patients coming to the retina clinic with efficient control of blood sugar without retinopathy and the others who have poor glycemic control with ME versus controls.

All statistical tests and analyses were based on the average data from the eyes. Data was analyzed using Statistical Package for the Social Sciences (SPSS) (SPSS Statistics is a statistical software suite developed by (International Business Machines Corporation) IBM for data management, is an American multinational technology company headquartered in Armonk, New York). The corneal descriptors' endothelial cell density (ECD), CV, HEX %, and CCT were obtained and expressed as mean  $\pm$  SD.

Ethical and juridical aspects of this research have been checked by the medical ethical committee.

### 2.3. Investigations

Imaging modality: all imaging modalities were done at the beginning of the study and at first month, second month, and third month after intravitreal injection. We underwent fluorescein angiography and optical coherence tomography on patients in group 1, and all the cases underwent a noncontact specular microscopy to assess the endothelium in the center of the cornea (Figs. 1–4).

Materials for analysis of the endothelial cell: a Topcon SP-1P noncontact specular microscope was used, and the corneal endothelium was examined under the microscope's specular lens, without touching the cornea. It offers good image quality, high magnification, and the ability to perform semiautomated, computer-assisted CD analysis, and morphometric analysis. The SP-1P robotically combines three photos – the central, nasal, and temporal – enabling us to observe the endothelial cells efficiently. We also analyzed the cell sizes, CCT, the CV of cell area (SD/mean), and the proportion of hexagonal cells. Approximately each image analysis had 100 cells counted in. The captured image was then analyzed with Topcon cell count software.

Laboratory investigation: glycosylated hemoglobin at the beginning of the study and after 3 months of intravitreal injection.

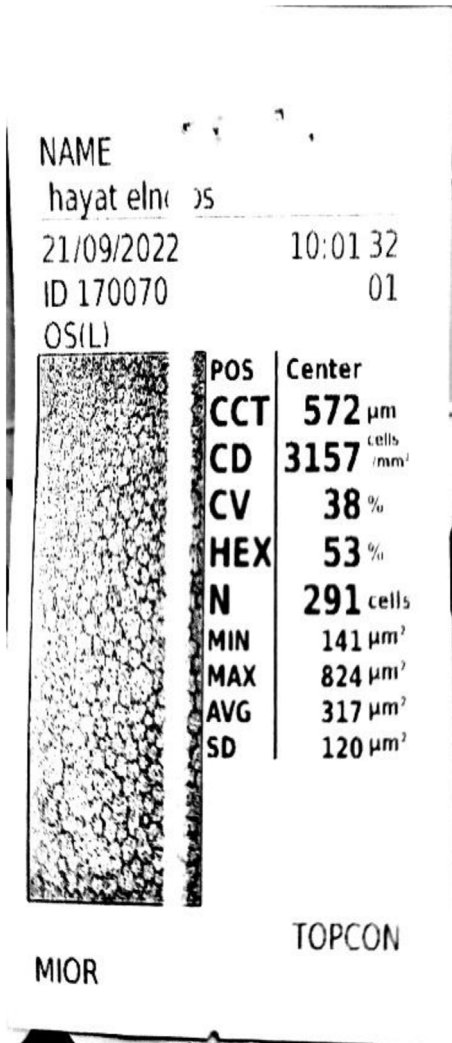


Fig. 1. Specular microscopy before the first injection.

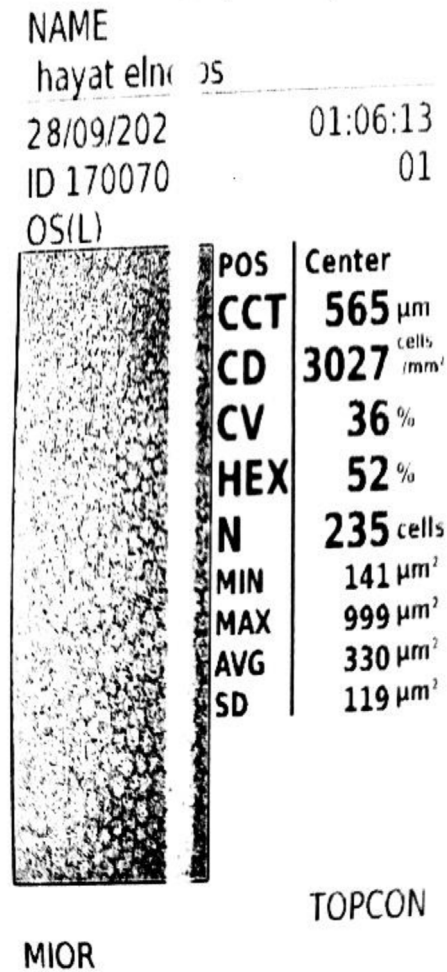


Fig. 2. Specular microscopy after the first injection.

Surgical intervention: patients in group 1 with DME underwent preoperative clinical evaluation and investigation. Then, they were injected intravitreally by (0.5 mg in 0.05 ml) of ranibizumab. At first, second, and third months after the first injection postoperative clinical evaluation and investigation were done as scheduled above.

The intravitreal injection technique of ranibizumab: 4% benoxinate was injected to cause topical anesthesia. With 0.027% iodine, we cleaned both the ocular surface and the eyelids. Using a 32-G needle, ranibizumab was injected in the operating room inside the vitreous cavity 4 mm behind the limbus in the infratemporal quadrant via the pars plana. The post-injection light perception was assessed. Before and after each intravitreal injection, a topical 0.5% levofloxacin ED was used four times per day for 3 days, while brimonidine ED was used two times per day for 3 days after the administration of ranibizumab.

### 3. Results

In our study, the average age in group 1 was  $60.40 \pm 4.99$ , while the average age in group 2 was  $58.70 \pm 4.27$ , while average age in group 3 was  $46.90 \pm 9.22$ , group 1 0.0% of them was males and 100.0% were females, while the group 2; 40.0% were males and 60.0% were females, while the group 3; 70.0% were males and 30.0% were females (Table 1).

We revealed that whereas ECD, size area, CV, in addition to the CCT, did not differ between DM patients and controls, DM patients had lower ECD than normal participants identical in age and sex. When compared to nondiabetic participants ( $2699.9 \pm 38.7 \text{ cells/mm}^2$ ), diabetics had a considerably lower CD ( $2577.2 \pm 27.3 \text{ cells/mm}^2$ ) ( $P = 0.01$ ) (Table 2).

Before therapy, patients getting injections of ranibizumab had an average ECD of  $2397 \pm 459 \text{ cells/mm}^2$  before treatment,

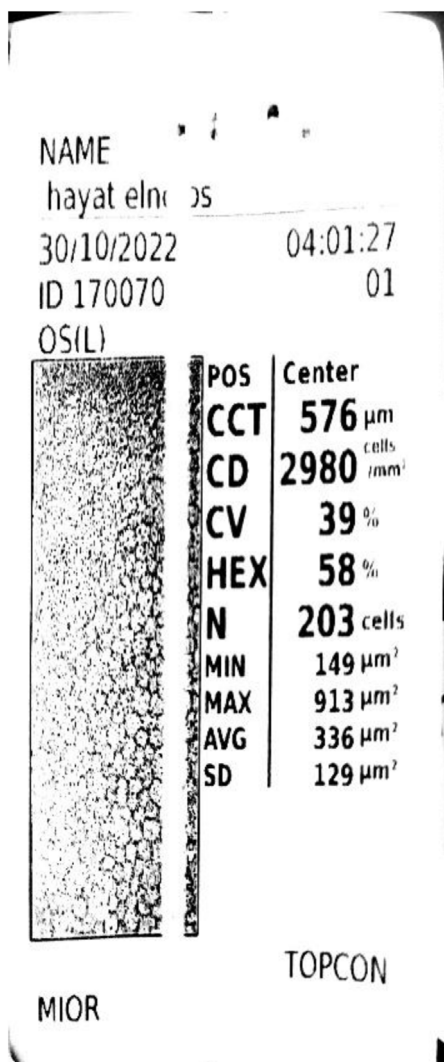


Fig. 3. Specular microscopy after the second injection.

2389  $\pm$  459 cells/mm<sup>2</sup> after the first injection, 2386  $\pm$  467 cells/mm<sup>2</sup> after the second injection, 2378  $\pm$  475 cells/mm<sup>2</sup> after the third injection, and 2357  $\pm$  460 cells/mm<sup>2</sup> 3 months following first ranibizumab.

ECD decreased by 0.3% following the first ranibizumab. Then decreased by about 0.5% following second ranibizumab, 0.8% following the third ranibizumab, and 1.7% 3 months following the first ranibizumab. In our patients, the average number of hexagonal cells (pleomorphism) was 53.7  $\pm$  9% before treatment, 51.6  $\pm$  8% following the first ranibizumab, 50.6  $\pm$  8% following the second ranibizumab, 49.8  $\pm$  9% following the third ranibizumab, and 49.2  $\pm$  9% 3 months following the first ranibizumab (Tables 3 and 4).

We found no changes in the CCT, count of endothelial cell, polymegathism, or pleomorphism following anti-VEGF(ranibizumab) injection by the

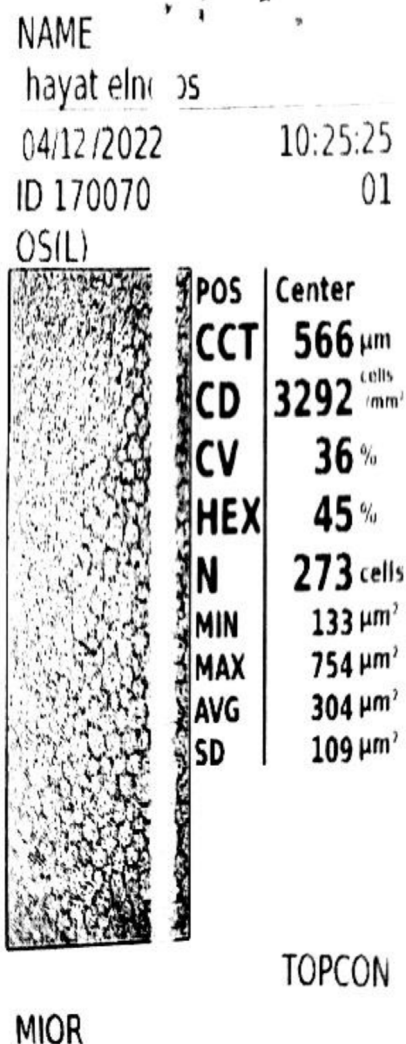


Fig. 4. Specular microscopy after the third injection.

dose of (0.5 mg/0.05 ml) intravitreal injection ranibizumab monthly for 3 months on the row as a therapy for DME, demonstrating the safety of anti-VEGF antibody use for the endothelium of the cornea in addition to the morphology of the cornea.

#### 4. Discussion

Diabetes can lead to DME, with vascular hyperpermeability and a tendency to accumulate fluid at the macula as a result of the defects in the blood–retinal barrier. VEGF is vital in the pathogenesis of DME. So, intravitreal anti-VEGF medications are currently the ‘primary way’ for treating

Table 1. Comparison between group 1 (N = 10), group 2 (N = 10), and group 3 (N = 10) regarding age (year), general condition, sex, general condition (NIDDM), and glycosylated hemoglobin.

	Group 1	Group 2	Group 3	Test value	P value	Significance
Age (year)						
Mean $\pm$ SD	60.40 $\pm$ 4.99	58.70 $\pm$ 4.27	46.90 $\pm$ 9.22	12.656 $\bullet$	0.000	HS
Range	50–65	51–63	35–61			
General condition [n (%)]						
Free	10 (100.0)	10 (100.0)	10 (100.0)	–	–	–
Sex [n (%)]						
Female	10 (100.0)	6 (60.0)	3 (30.0)	10.622*	0.005	HHS
Male	0	4 (40.0)	7 (70.0)			
OD [n (%)]	6 (60.0)	4 (40.0)	50 (50.0)	0.800*	0.670	NNS
OS [n (%)]	4 (40.0)	6 (60.0)	50 (50.0)			
General condition (NIDDM)						
Mean $\pm$ SD	3.90 $\pm$ 1.10	4.90 $\pm$ 0.99	–	4.545*	0.047	SS
Range	2–5	3–6	–			
HbA1c						
Mean $\pm$ SD	7.50 $\pm$ 2.22	5.65 $\pm$ 0.88	4.80 $\pm$ 0.17	9.937*	0.001	HHS
Range	5–13	4.5–7	4.50–5			

HbA1c, glycosylated hemoglobin.

\* Chi-square test.

 $\bullet$  One way ANOVA test.Table 2. Comparison between group 1 (N = 10), group 2 (N = 10), and group 3 (N = 10) regarding central corneal thickness ( $\mu$ M), CD (cell/ $\text{mm}^2$ ), coefficient of variation (%), and hexagonality (%).

Pre 1st inj. Info	Group 1	Group 2	Group 3	Test value*	P value	Significance
CCT ( $\mu$ M)						
Mean $\pm$ SD	547.50 $\pm$ 46.56	541.40 $\pm$ 25.82	509.10 $\pm$ 17.15	4.083	0.028	S
Range	498–635	496–570	478–535			
CD (cell/ $\text{mm}^2$ )						
Mean $\pm$ SD	2566.80 $\pm$ 590.39	2962.00 $\pm$ 316.96	2798.50 $\pm$ 302.40	2.189	0.132	NS
Range	1906–3761	2398–3387	2377–3205			
CV (%)						
Mean $\pm$ SD	32.30 $\pm$ 6.18	37.60 $\pm$ 1.17	32.40 $\pm$ 2.88	5.758	0.008	HS
Range	21–43	36–40	28–37			
HEX (%)						
Mean $\pm$ SD	54.60 $\pm$ 17.51	33.00 $\pm$ 4.06	45.40 $\pm$ 10.30	8.217	0.002	HS
Range	15–74	24–38	31–58			

CCT, central corneal thickness; CD, coefficient of variation; CV, coefficient of variation; HEX, hexagonality.

\* One way ANOVA test.

Table 3. Comparison between pre first injection, post first injection, post second injection, and post third injection regarding central corneal thickness ( $\mu$ M), group 1.

CCT ( $\mu$ M)	Group 1 (N = 10)	Test value $^{\text{E}}$	P value	Significance
Pre 1st inj				
Mean $\pm$ SD	547.50 $\pm$ 46.56	–	–	–
Range	498–635			
Post				
1st inj.				
Mean $\pm$ SD	546.80 $\pm$ 51.11	0.218	0.832	NS
Range	480–636			
2nd inj.				
Mean $\pm$ SD	543.70 $\pm$ 52.57	0.924	0.380	NS
Range	482–637			
3rd inj.				
Mean $\pm$ SD	541.20 $\pm$ 52.53	1.402	0.194	NS
Range	480–635			

CCT, central corneal thickness.

 $^{\text{E}}$  Paired t-test.

Table 4. Comparison between pre first injection, post first injection, post second injection and post third injection regarding central corneal thickness (μM), group 2.

CCT (μM)	Group 2 (N = 10)	Test value <sup>£</sup>	P value	Significance
Pre 1st inj				
Mean ± SD	541.40 ± 25.82	–	–	–
Range	496–570			
Post	–	–	–	
1st inj.				
Mean ± SD	547.30 ± 25.22	–2.343	0.044	S
Range	506–574			
2nd inj.				
Mean ± SD	548.10 ± 24.93	–2.590	0.029	S
Range	508–575			
3rd inj.				
Mean ± SD	549.10 ± 25.21	–2.967	0.016	S
Range	509–577			

CCT, central corneal thickness.

<sup>£</sup> Paired t-test.

DME, and extensive randomized clinical trials have supported their efficacy.<sup>5</sup>

It has been discovered that anti-VEGF compounds attach to VEGF receptors to block the VEGF signal, preventing the creation of aberrant blood vessels and reducing vascular permeability.<sup>6</sup> The corneal epithelium, endothelium, and stroma contain VEGF and its receptors, hence anti-VEGF therapy may have an impact on the cornea, as has been shown in cases of bevacizumab-treated macular degeneration that result from growing old in the past.<sup>7</sup>

Ranibizumab is a fragment that comes from a human anti-VEGF antibodies, can bind all VEGF patterns, and is created using recombinant monoclonal antibody technology.<sup>8</sup>

Guzel *et al.*<sup>9</sup> looked into how intravitreal ranibizumab affected the endothelial layer of the cornea during the treatment course of DME. They found that 3 months of monthly injections of either ranibizumab had no hazardous on the endothelial cells or on corneal morphology.

ECD was shown to be lesser in DM candidates compared to nondiabetic individuals. Therefore, patients with DME may experience substantial stress from intravitreal injections due to their already weakened endothelial cells.<sup>10</sup>

In a recent study employing intravitreal injections to treat DME or cystoid ME brought on by blockage of the retinal vein, it had been discovered that aflibercept generated a small alteration in the endothelial cells of the cornea in 6 months of the initial treatment. However, minimal variation in ECD, CCT, or HEX was seen following the treatment.<sup>11</sup>

Some studies found that the endothelial layer of the cornea and the corneal morphology and CCT did not change after injection of 2.5 mg bevacizumab for 6 months as a single dose.<sup>6</sup>

Additionally, for corneal neovascularization treatment, Lichtinger *et al.*<sup>12</sup> discovered the minimal difference in parameters of CD, CV, HEX, and CCT between baseline with 6 months following three simultaneous subconjunctival bevacizumab injections. Another study found the absence of negative effects of bevacizumab on CCT or CD, indicating that it is safe in treating such conditions.<sup>13</sup>

Pérez-Rico *et al.*<sup>14</sup> examined the effects of three successive monthly doses of 0.5 mg/0.05 ml intravitreal injection ranibizumab on the count of endothelial cells, the thickness of the central part of the cornea, and polymegathism for treating choroidal neovascularization in macular degeneration brought on by aging but found no evidence of a significant difference.

For iris neovessels treatment, Hosny *et al.*<sup>15</sup> found that injection of 1.25 mg/0.05 ml of bevacizumab intravitreally did not change the structure or the morphology of corneal endothelium layer.

Scanning electron microscopy was used by Ari *et al.*<sup>16</sup> to investigate the impact of intracameral ranibizumab on the rabbit corneal endothelium. Following the intracameral administration of ranibizumab 1 and 0.5 mg, they noticed a deterioration in endothelial cell morphology. Aflibercept and ranibizumab, two different anti-VEGF medications, have been shown by Gharbiya *et al.*<sup>17</sup> to change the survival and death of the rabbit corneal endothelium.

In the current investigation, we also examined the CCT. Before therapy, CCT in ranibizumab-treated individuals was  $551.1 \pm 34$  μm. After the second ranibizumab ( $555.7 \pm 35$  μm) and 6 months after the first ranibizumab ( $555.36$  μm), there was a significant rise in CCT. Compared to the CCT readings obtained before treatment, just 4 μm thicker was the

final CCT in this patient group. After receiving aflibercept injection, the CCT of the patients receiving aflibercept injection did not alter significantly from baseline. Additional writers found comparable outcomes.<sup>18</sup>

The cornea, for example, has VEGF, VEGF-1, and VEGF-2 receptors, but Yoeruek *et al.*<sup>19</sup> found that maintaining fms-related tyrosine kinase 1 making a balance prevented corneal avascularization. After receiving a bevacizumab injection, Bayar and colleagues noticed condensed corneal edema in a series of five cases, which may have been caused by VEGF in limbal vessels inhibiting its ability to activate the immune system. However, no corneal endothelium abnormalities were seen, and edema of the cornea was always cured within 10 days with steroids and antibiotics. But, their research still suggests that bevacizumab may result in morphological and immunological modifications in the endothelium of the cornea.<sup>10</sup>

The epithelium, stroma, and endothelium of the cornea generally release VEGF. However, it is also, to a lesser extent, secreted by limbal vessels and keratocytes. In pathological situations, newly formed stromal vessels can secrete significant quantities of it. VEGF receptors have been found to exist in the corneal endothelium in experimental testing. It was noted that VEGF levels in the aqueous humour reduced at least 10 times after injection of ranibizumab intravitreally 1 week later, in addition to 29 days after the injection, it was easily to discover anti-VEGF antibodies in the aqueous humour.<sup>3</sup>

In addition, Philipp and colleagues detected VEGF expression in human corneal epithelial, endothelial, and faintly keratocyte cells. On the endothelial cells of the cornea, Flk-1/KDR expression was not present, while Flt-1 expression was just weakly present. Additionally, in that research, it was found in inflamed and vascularized human corneas, the VEGF and its receptors were overexpressed, indicating the role of angiogenic cytokine and the pathophysiology of neovascularization of the cornea.<sup>20</sup>

In an in-vitro study, Yoeruek *et al.*<sup>19</sup> revealed that VEGF, VEGFR1, and VEGFR2 immunoreactivity was present in all corneal cells in humans. VEGF's paradoxical presence in avascular tissue, like the cornea, must be understood. In light of this, we believe ranibizumab injections in the aqueous humour could influence VEGF activity in the corneal endothelium.

After three consecutive monthly intravitreal injections, it was found in the current study that ranibizumab significantly improved central macular

thickness (CMT). This improvement persisted for 3 months after the final injection. These conclusions are supported by the findings of earlier research on the association between intravitreal ranibizumab and visual acuity or macular thickness.<sup>21</sup>

According to previous large clinical trials, visual acuity gains over baseline seemed connected with CMT declines over baseline.<sup>22</sup> There was a substantial ( $P<0.01$ ) association between the mean change in the mean change in CMT throughout all research stages. The link between CMT and visual acuity following ranibizumab is supported by these findings, consistent with those of earlier investigations.<sup>21</sup>

The baseline CMT and 1 month after the first injection were found to have the most significant correlation in the current study among the three injections. According to a report, the baseline CMT may be able to predict how structures will respond to intravitreal ranibizumab.<sup>23</sup>

All patients in this study suffered from DME only and were followed up for 3 months. Similar to the duration of the follow-up in Chiang *et al.*<sup>6</sup>

In addition to the forecast provided by the initial CMT, to forecast the functional and structural outcomes of an intravitreal ranibizumab injection in patients with DME, the study team hypothesized that it might be helpful to measure the efficacy as soon as 1 month after the injection. Chatziralli *et al.*<sup>4</sup> conducted a study. In their study, the follow-up period was 12 months.

In our study, there were three injections with 3-month duration of follow-ups; in the most of other studies, there was only one injection and multiple follow-ups have been done for this solely intervention.

The corneal endothelial cell morphology appears unaffected by the administration of anti-VEGF injections containing ranibizumab, 0.5 mg/0.05 ml.

Several limitations exist in our study. First, the study had a relatively small sample size. Second, the follow-up period was limited to just 3 months all over the course of injections, potentially missing longer-term effects or complications. Third, the absence of a placebo or sham injection control group raises challenges in attributing observed changes solely to ranibizumab injections. Additionally, the study included diabetic patients with varying characteristics, such as retinopathy status and glycemic control, introducing potential confounding variables. Furthermore there was no comparison with other anti-VEGF agents commonly used in DME. Being a single-center study further restricts the generalizability of our findings. Although the study suggested that ranibizumab was



safe for corneal endothelium and morphology, a more comprehensive safety assessment, including ocular and systemic adverse events, would be necessary for definitive conclusions. Lastly, while significant improvements were observed in best-corrected visual acuity and CMT up to 3 months after the last injection, longer-term effects on these parameters were not explored.

#### 4.1. Conclusion

Injection of anti-VEGF intravitreally in DME patients showed no corneal endothelium or CCT changes after receiving ranibizumab 0.5 mg/0.05 ml. Therefore, the safety of intravitreal anti-VEGF injections has been determined for the corneal endothelium. Extra researches are needed.

#### Consent statement

All patients have been informed about the details and provided a written consent. It is included in patients' profile and ticket in the hospital where the procedure has been done.

#### Conflicts of interest

None declared.

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