Assessment of Retinal Capillary Blood Flow, Volume and Velocity Before and After Intravitreal Ranibizumab or Triamcinolone Acetonide Injection in Diabetic Macular Edema

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Abstract: Diabetic retinopathy (DR) and diabetic macular edema (DME), serious eye conditions caused primarily by hyperglycemia, are the major cause of loss of vision and blindness in the working population of developed countries. The pathogenesis of DME has not been fully elucidated since it is caused by complex pathological process with many contributing factors. Dysfunction of the inner and outer retinal barriers leads to accumulation of sub- and intra-retinal fluid in the inner- and outer-plexiform layers. Vascular endothelial growth factor (VEGF) has generally been accepted as the main factor that disrupts the inner blood-retinal barrier (BRB) function, making it an important target for pharmaceutical intervention. Disturbance of retinal capillary blood flow is feature of many ocular diseases, including diabetic retinopathy. A number of non-invasive instruments have been designed to measure retinal hemodynamics. Heidelberg retinal Flowmeter (HRF) is unique in that as is provides a two dimensional quantifiable perfusion map of retinal capillary blood flow rather than a measurement of flow at a single point. HRF has potential as a non-invasive clinical tool that visualize and quantify retinal capillary blood flow. OCT is a non invasive and non contact diagnostic method introduced in 1995 for imaging macular diseases. OCT is valuable diagnostic tool in DME, helpful in both diagnostic and follow up procedures. There are different approaches for the treatment: anti-VEGF, steroids, laser, and vitrectomy which play important roles in the management of DME. In the present study assessment of the retinal capillary blood flow, volume and velocity before and after intrvitreal Ranibizumab or Triamcinolone acetonide injection in diabetic macular edema was done using HRF. The study was done in Sayed Galal hospital and included 75 patients, 25 of them were normal individuals, 25 injected with ranibizumab and 25 injected with triamicinolone acetonide. The retinal capillary blood flow, volume and velocity are measured using HRF before and after injection of ranibizumab and triamicinolone acetonide in diabetic macular oedema. The retinal blood flow is elevated in diabetic patient before injection and was reduced following injection in both groups, with no statistically significant difference. The retinal blood volume is elevated in diabetic patient before and after injection and was reduced following injection in both groups, with no statistically significant difference. The retinal blood velocity is elevated and was reduced following injection in both groups, with no statistically significant difference. Concerning the visual acuity, it improved significantly in both groups following injection with no statistically significant difference between ranibizumab and triamicinolone acetonide. The central retinal thickness improved significantly in both groups following injection as measured by OCT with no statistically significant difference between both groups. However, in our current study there is a statistically significant increase in IOP following triamicinolone acetonide injection (18 eye, 72%) while there is no significant change in IOP following ranibizumab injection. So it is preferred to treat diabetic macular oedema with ranibizumab rather than triamicinolone acetonide.

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1. Introduction

Diabetic retinopathy (DR) and diabetic macular edema (DME), serious eye conditions caused primarily by hyperglycemia, are the major cause of loss of vision and blindness in the working population of developed countries. [1]

The pathogenesis of DME has not been fully elucidated since it is caused by complex pathological process with many contributing factors. Dysfunction of the inner and outer retinal barriers leads to accumulation of sub- and intra-retinal fluid in the inner- and outer-plexiform layers. Vascular endothelial growth factor (VEGF) has generally been accepted as the main factor that disrupts the inner blood-retinal barrier (BRB) function, making it an important target for pharmaceutical intervention. [2]

Hypoxia, ischemia, oxygen-free radicals and inflammatory mediators are all involved in the breakdown of retinal blood barrier (BRB). Muller cell, pericyte and glial cell dysfunction combined with

vitreous changes are involved in the occurrence and development of macular edema. Chronic hyperglycemia, hypertension and high cholesterol are also important factors related to the incidence of macular edema. [3]

DME is clinically classified as diffuse, focal or both. DME is characterized by microaneurysm formation and diffuse leakage from the retinal capillaries or arterioles. [4]

In the past, the term "clinically significant macular edema" (CSME) was used to define patients who needed to be treated. CSME was identified in the presence of any of the following three fundoscopic examination findings: [5]

1. Thickening of the retina at or within 500 microns of the center of the macula.
2. Hard exudates at or within 500 microns of the center of the macula, if associated with thickening of adjacent retina (excluding residual hard exudates remaining after disappearance of retinal thickening).
3. Retinal thickening at one disc area or larger, at any part of which is within one disc diameter of the center of the macula.

The optical coherence tomography (OCT), is a noninvasive and noncontact diagnostic method, was introduced 1995 for imaging macular diseases. In diabetic macular edema, OCT scans show hyporeflectivity, due to intraretinal and subretinal fluid accumulation, related to inner and outer blood retinal barrier breakdown. OCT tomograms may also reveal the presence of hard exudates, as hyperreflective spots with a shadow, in the outer retinal layers. Among other methods OCT is a particularly valuable diagnostic tool is DME, helpful in both diagnostic and follow up procedures. [6]

Disturbance of retinal capillary blood flow is feature of many ocular diseases, including diabetic retinopathy. A number of non-invasive instruments have been designed to measure retinal hemodynamics. Heidelberg retinal Flowmeter (HRF) is unique in that as is provides a two dimensional quantifiable perfusion map of retinal capillary blood flow rather than a measurement of flow at a single point. HRF has potential as a non-invasive clinical tool that visualize and quantify retinal capillary blood flow. [7]

OCT angiography permits the noninvasive imaging of retinal and choroidal circulation via motion contrast imaging. This relatively novel imaging technique obtains high-resolution volumetric blood flow information and generates angiographic images in a matter of seconds. OCT angiograms are resampled with OCT B-scans from the same area, simultaneously allowing the assessment of structure and blood flow. [8]

The Early Treatment Diabetic Retinopathy Study (ETDRS) provided a treatment paradigm in this disease using laser therapy to reduce moderate vision loss in patients with clinically significant macular edema by approximately 50%, although prevention of vision loss is important, visual improvement would be preferable. There are different approaches for the treatment: anti-VEGF, steroids, laser, and vitrectomy which play important roles in the management of DME. [9]

Aim of the Work

To measure the retinal capillary blood flow, volume and velocity at the macula and juxta papillary area before and after intravitreal triamcinolone acetonide or intravitreal Ranibizumab injection in diabetic macular edema using HRF imaging.

2. Patients and Methods

Study design

A randomized interventional case-controlled study that included seventy five eyes of fifty patients, to compare the visual acuity, OCT changes and retinal capillary blood flow using HRF. The study was carried out from May 2015 to March 2017.

The patients were selected from the outpatient Ophthalmic Clinic of Al-Azhar hospitals and Memorial institute of ophthalmic research. The protocol was revised and approved by Al-Azhar University Ophthalmology Ethical Committee; informed written consent was obtained from all patients before the initiation of any procedure.

Patient selection:

Inclusion criteria

- Diabetic patients
- Clinically significant macular edema
- Non-ischemic macular edema
- Macular thickness more than 250 microns
- Age from 30-70 years

Exclusion criteria

- Systemic diseases other than diabetes as hypertension and chronic renal failure.
- Tractional retinal detachment
- Proliferative diabetic retinopathy
- Ischemic macular edema
- Macular thickness less than 250 microns
- High refractive errors and media opacity as corneal opacities and cataract
- Macular degeneration
- Previous laser photocoagulation treatment
- Intraocular pressure more than 21 mmHg

Treatment groups:

The patients are divided into 3 groups:

Group A: 25 eyes for normal individuals.

Group B: 25 eyes are injected by Ranibizumab (leucentis).

Group C: 25 eyes are injected by Triamcinolone Acetonide.

Preoperative evaluation:

- Best corrected visual acuity is measured using Snellen chart.
- Slit lamp examination of the anterior segment is done to exclude any media opacity as corneal opacity and cataract.
- IOP measurement to exclude glaucomatous patients. This is done by Goldman applanation tonometer.
- Fundus examination is done using 20D and 90D lens to exclude any retinal condition that may affect retinal blood flow as retinal detachment.
- Fundus flourescien angiography to diagnose macular edema is done.
- OCT is done to measure the central macular thickness.
- HRF is used to measure retinal capillary blood flow.
- Systemic examination:

i. Blood pressure measurement.
ii. Blood sugar analysis.

Methods:

Measurement of blood flow using HRF is done above the lower temporal arcade and below the upper temporal arcade and the macular area.

All groups underwent the following:

- Dilatation of patient pupil is not required for recording image data, pupil diameter of 1mm was found to be sufficient to receive useful data.
- Adjustment of the camera to eye being examined.
- Operation panel is adjusted as follows:

1. Focal planes to the refraction of examined eye (spherical equivalent)
2. Scan depth.
3. Size of scanning field.
4. Laser intensity setting.

- Positioning of head and chin of the patient firmly against the head and chin rest.
- Acquiring three images of the upper and lower arcade.
- Processing of the images.
- Measurement of blood flow, volume and velocity were recorded at examined area.

Postoperative follow up

First day postoperatively:

Complete ophthalmic examination:

- Best corrected visual acuity is measured.
- Slit lamp examination.
- Applanation tonometry is done to measure IOP postoperatively.
- Fundus examination.

One month postoperatively:

Complete ophthalmic examination:

- Best corrected visual acuity is measured.
- Slit lamp examination.
- Applanation tonometry is done to measure IOP postoperatively.
- Fundus examination.
- OCT is done to assess macular thickness following the injection.
- Measurement of blood flow, volume and velocity using HRF one month postoperatively.

Statistical Analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric. Also qualitative variables were presented as number and percentages.

The comparison between groups regarding qualitative data was done by using **Chi-square test** and/or **Fisher exact test** only when the expected count in any cell found less than 5.

The comparison between two independent groups regarding quantitative data with parametric distribution was done by using **Independent t-test.**

The comparison between more than two independent groups regarding quantitative data with parametric distribution was done by using **One Way ANOVA.**

The comparison between two paired groups regarding quantitative data with parametric distribution was done by using **Paired t-test.**

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

$P > 0.05$: Non significant (NS).

$P < 0.05$: Significant (S).

$P < 0.01$: Highly significant (HS).

3. Results

Table (1): Comparison between the three studied groups regarding age and sex of the studied cases

		Group A No. = 25	Group B No. = 25	Group C No. = 25	Test value	P-value	Sig.
Sex	Females Males	13 (52.0%) 12 (48.0%)	10 (40.0%) 15 (60.0%)	11 (44.0%) 14 (56.0%)	0.753	0.686	NS
Age (years)	Mean±SD Range	59.52 ± 6.82 42 – 69	56.15 ± 7.82 40 – 67	55.28 ± 7.48 38 – 70	0.161	0.852	NS

The previous table shows that there was no statistically significant difference found between the three studied groups regarding sex and age of the studied cases with p-value = 0.686 and 0.852 respectively.

Table (2): Comparison between the group A and group B regarding upper and lower retinal blood flow of the studied cases pre injection

Pre injection		Group A No. = 25	Group B No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	230.7 ± 25.9 180 – 289	318.5 ± 31.4 273 – 383	10.785	<0.001	HS
Lower	Mean±SD Range	225.3 ± 18.7 165 – 270	309.8 ± 23.5 251 – 367	14.068	<0.001	HS

The previous table shows that there was statistically significant difference between group A and group B regarding retinal blood flow pre injection upper and lower.

Table (3): Comparison between the group A and group C regarding upper and lower retinal blood flow of the studied cases pre injection.

Pre injection		Group A No. = 25	Group C No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	230.7 ± 25.9 180 – 289	303.24 ± 29.26 261 – 369	9.282	<0.001	HS
Lower	Mean±SD Range	225.3 ± 18.7 165 – 270	295.2 ± 31.72 231 – 342	9.492	<0.001	HS

The previous table shows that there was statistically significant difference between group A and group C regarding retinal blood flow pre injection upper and lower.

Table (4): Comparison between the group B and group C regarding upper and lower retinal blood flow of the studied cases pre injection

Pre injection		Group B No. = 25	Group C No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	318.5 ± 31.4 273 – 383	303.24 ± 29.26 261 – 369	1.778	0.082	NS
Lower	Mean±SD Range	309.8 ± 23.5 251 – 367	295.2 ± 31.72 231 – 342	1.849	0.071	NS

The previous table shows that there was no statistically significant difference between group B and group C regarding retinal blood flow pre injection upper and lower.

Table (5): Comparison between the group A and group B regarding upper and lower retinal blood flow of the studied cases post injection

Post injection		Group A No. = 25	Group B No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	230.7 ± 25.9 180 – 289	233.48 ± 27.68 191 – 306	0.367	0.715	NS
Lower	Mean±SD Range	225.3 ± 18.7 165 – 270	231.28 ± 26.33 183 – 297	0.926	0.359	NS

The previous table shows that there was no statistically significant difference between group A and group B regarding upper and lower retinal blood flow post injection.

Table (6): Comparison between the group A and group C regarding upper and lower retinal blood flow of the studied cases post injection.

Post injection		Group A No. = 25	Group C No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	230.7 ± 25.9 180 – 289	224.28 ± 23.03 175 – 279	0.926	0.359	NS
Lower	Mean±SD Range	225.3 ± 18.7 165 – 270	218.52 ± 24.69 192 – 286	1.095	0.279	NS

The previous table shows that there was no statistically significant difference between group A and group C regarding upper and lower retinal blood flow post injection.

Table (7): Comparison between the group B and group C regarding upper and lower retinal blood flow of the studied cases post injection.

Post injection		Group B No. = 25	Group C No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	233.48 ± 27.68 191 – 306	224.28 ± 23.03 175 – 279	1.278	0.208	NS
Lower	Mean±SD Range	231.28 ± 26.33 183 – 297	218.52 ± 24.69 192 – 286	1.768	0.084	NS

The previous table shows that there was no statistically significant difference between group B and group C regarding upper and lower retinal blood flow post injection

Table (8): Comparison between the pre and post injection regarding retinal blood flow in group B

Group B		Pre injection No. = 25	Post injection No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	318.5 ± 31.4 273 – 383	233.48 ± 27.68 191 – 306	7.405	<0.001	HS
Lower	Mean±SD Range	309.8 ± 23.5 251 – 367	231.28 ± 26.33 183 – 297	9.207	<0.001	HS

The previous table shows that there was statistically significant decrease in retinal blood flow in group B post injection than pre injection.

Table (9): Comparison between the pre and post injection regarding retinal blood flow in group C

Group C		Pre injection No. = 25	Post injection No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	303.24 ± 29.26 261 – 369	224.28 ± 23.03 175 – 279	6.570	<0.001	HS
Lower	Mean±SD Range	295.2 ± 31.72 231 – 342	218.52 ± 24.69 192 – 286	8.930	<0.001	HS

The previous table shows that there was statistically significant decrease in retinal blood flow in group C post injection than pre injection.

Table (10): Comparison between the group A and group B regarding upper and lower retinal blood volume of the studied cases pre injection

Volume pre injection		Group A	Group B	Test value	P-value	Sig.
Upper	Mean±SD Range	17.48 ± 2.20 15 – 24	27.17 ± 3.45 21 – 39	11.841	< 0.001	HS
Lower	Mean±SD Range	16.84 ± 1.91 13 – 21	26.51 ± 2.31 19 – 36	16.131	< 0.001	HS

The previous table shows that there was statistically significant difference between group A and B regarding retinal blood volume pre and post injection.

Table (11): Comparison between the group A and group C regarding upper and lower retinal blood volume of the studied cases pre injection

Volume pre injection		Group A	Group C	Test value	P-value	Sig.
Upper	Mean±SD Range	17.48 ± 2.20 15 – 24	26.8 ± 2.85 22 – 37	12.943	<0.001	HS
Lower	Mean±SD Range	16.84 ± 1.91 13 – 21	25.9 ± 2.12 20 – 38	15.875	<0.001	HS

The previous table shows that there was statistically significant difference between group A and C regarding retinal blood volume pre and post injection.

Table (12): Comparison between the group B and group C regarding upper and lower retinal blood volume of the studied cases pre injection

Volume pre injection		Group B	Group C	Test value	P-value	Sig.
Upper	Mean±SD Range	27.17 ± 3.45 21 – 39	26.8 ± 2.85 22 – 37	0.413	0.681	NS
Lower	Mean±SD Range	26.51 ± 2.31 19 – 36	25.9 ± 2.12 20 – 38	0.973	0.335	NS

The previous table shows that there was no statistically significant difference between group B and C regarding retinal blood volume pre and post injection.

Table (13): Comparison between the group A and group B regarding upper and lower retinal blood volume of the studied cases post injection

Volume post injection		Group A	Group B	Test value	P-value	Sig.
Upper	Mean±SD Range	17.48 ± 2.20 15 – 24	19.1 ± 4.25 17 – 27	1.693	0.097	NS
Lower	Mean±SD Range	16.84 ± 1.91 13 – 21	18.3 ± 3.71 15 – 28	1.749	0.087	NS

The previous table shows that there was no statistically significant difference between group A and B regarding upper and lower retinal blood volume post injection.

Table (14): Comparison between the group A and group C regarding upper and lower retinal blood volume of the studied cases post injection

Volume post injection		Group A	Group C	Test value	P-value	Sig.
Upper	Mean±SD Range	17.48 ± 2.20 15 – 24	18.21 ± 3.51 16 – 25	0.881	0.383	NS
Lower	Mean±SD Range	16.84 ± 1.91 13 – 21	18.6 ± 4.20 17 – 29	1.907	0.063	NS

The previous table shows that there was no statistically significant difference between group A and C regarding upper and lower retinal blood volume post injection.

Table (15): Comparison between the group B and group C regarding upper and lower retinal blood volume of the studied cases post injection

Volume post injection		Group B	Group C	Test value	P-value	Sig.
Upper	Mean±SD Range	19.1 ± 4.25 17 – 27	18.21 ± 3.51 16 – 25	0.807	0.423	NS
Lower	Mean±SD Range	18.3 ± 3.71 15 – 28	18.6 ± 4.20 17 – 29	0.268	0.790	NS

The previous table shows that there was no statistically significant difference between group B and C regarding upper and lower volume post injection.

Table (16): Comparison between retinal blood volume pre and post injection in group B

Group B		Pre injection No. = 25	Post injection No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	27.17 ± 3.45 21 – 39	19.1 ± 4.25 17 – 27	9.412	<0.001	HS
Lower	Mean±SD Range	26.51 ± 2.31 19 – 36	18.3 ± 3.71 15 – 28	9.604	<0.001	HS

The previous table shows that there was highly statistically significant decrease in retinal blood volume post injection than pre injection in group B with p-value < 0.001.

Table (17): Comparison between retinal blood volume pre and post injection in group C

Group C		Pre injection No. = 25	Post injection No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	26.8 ± 2.85 22 – 37	18.21 ± 3.51 16 – 25	9.201	<0.001	HS
Lower	Mean±SD Range	25.9 ± 2.12 20 – 38	18.6 ± 4.20 17 – 29	9.031	<0.001	HS

The previous table shows that there was highly statistically significant decrease in retinal blood volume post injection than pre injection in group C with p-value < 0.001.

Table (18): Comparison between the group A and group B regarding upper and lower retinal blood velocity of the studied cases pre injection

Velocity pre injection		Group A	Group B	Test value	P-value	Sig.
Upper	Mean±SD Range	34.32 ± 2.29 29 – 38	38.60 ± 3.62 34 – 50	4.996	<0.001	HS
Lower	Mean±SD Range	34.28 ± 2.48 31 –39	41.88 ± 3.28 37 – 48	9.241	<0.001	HS

The previous table shows that there was statistically significant difference between group A and B regarding upper and lower velocity pre injection.

Table (19): Comparison between the group A and group C regarding upper and lower retinal blood velocity of the studied cases pre injection

Velocity pre injection		Group A	Group C	Test value	P-value	Sig.
Upper	Mean±SD Range	34.32 ± 2.29 29 – 38	39.84 ± 2.23 36 – 46	8.635	<0.001	HS
Lower	Mean±SD Range	34.28 ± 2.48 31 –39	39.92 ± 3.96 35 – 43	6.035	<0.001	HS

The previous table shows that there was statistically significant difference between group A and C regarding upper and lower velocity pre injection.

Table (20): Comparison between the group B and group C regarding upper and lower retinal blood velocity of the studied cases pre injection

Velocity pre injection		Group B	Group C	Test value	P-value	Sig.
Upper	Mean±SD Range	38.60 ± 3.62 34 – 50	39.84 ± 2.23 36 – 46	1.458	0.151	NS
Lower	Mean±SD Range	41.88 ± 3.28 37 – 48	39.92 ± 3.96 35 – 43	1.906	0.063	NS

The previous table shows that there was no statistically significant difference between group B and C regarding upper and lower velocity pre injection.

Table (21): Comparison between the group A and group B regarding upper and lower retinal blood velocity of the studied cases post injection

Velocity post injection		Group A	Group B	Test value	P-value	Sig.
Upper	Mean±SD Range	34.32 ± 2.29 29 – 38	33.94 ± 2.84 31 – 42	0.521	0.605	NS
Lower	Mean±SD Range	34.28 ± 2.48 31 –39	35.60 ± 2.50 33 – 40	1.874	0.067	NS

The previous table shows that there was no statistically significant difference between group A and B regarding upper and lower retinal blood velocity post injection.

Table (22): Comparison between the group A and group C regarding upper and lower retinal blood velocity of the studied cases post injection

Velocity post injection		Group A	Group C	Test value	P-value	Sig.
Upper	Mean±SD Range	34.32 ± 2.29 29 – 38	35.16 ± 2.85 32 – 44	1.149	0.256	NS
Lower	Mean±SD Range	34.28 ± 2.48 31 –39	36.02 ± 3.82 34 – 39	1.910	0.062	NS

The previous table shows that there was no statistically significant difference between group A and C regarding upper and lower retinal blood velocity post injection.

Table (23): Comparison between the group B and group C regarding upper and lower retinal blood velocity of the studied cases post injection

Velocity post injection		Group B	Group C	Test value	P-value	Sig.
Upper	Mean±SD Range	33.94 ± 2.84 31 – 42	35.16 ± 2.85 32 – 44	1.516	0.136	NS
Lower	Mean±SD Range	35.60 ± 2.50 33 – 40	36.02 ± 3.82 34 – 39	0.460	0.647	NS

The previous table shows that there was no statistically significant difference between group B and C regarding upper and lower retinal blood velocity post injection.

Table (24): Comparison between retinal blood velocity pre and post injection in group B

Group B		Pre injection No. = 25	Post injection No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	38.60 ± 3.62 34 – 50	33.94 ± 2.84 31 – 42	6.712	<0.001	HS
Lower	Mean±SD Range	41.88 ± 3.28 37 – 48	35.60 ± 2.50 33 – 40	9.568	<0.001	HS

The previous table shows that there was highly statistically significant decrease in retinal blood velocity post injection than pre injection in group B with p-value < 0.001.

Table (25): Comparison between retinal blood velocity pre and post injection in group C

Group C		Pre injection No. = 25	Post injection No. = 25	Test value	P-value	Sig.
Upper	Mean±SD Range	39.84 ± 2.23 36 – 46	35.16 ± 2.85 32 – 44	6.924	<0.001	HS
Lower	Mean±SD Range	39.92 ± 3.96 35 – 43	36.02 ± 3.82 34 – 39	4.722	<0.001	HS

The previous table shows that there was highly statistically significant decrease in retinal blood velocity post injection than pre injection in group B with p-value < 0.001.

Table (26): Comparison between group A and group B regarding IOP pre and post injection

		Group A	Group B	Test value	P-value	Sig.
IOP pre	Mean±SD Range	14.75 ± 1.62 13 – 17	14.52 ± 1.94 12 – 18	0.455	0.651	NS
IOP post	Mean±SD Range	14.75 ± 1.62 13 – 17	14.84 ± 1.82 12 – 19	0.185	0.854	NS
Paired t-test	t p-value		1.317 0.200 (NS)			

The previous table shows that there was no statistically significant difference found between group A and group B regarding IOP pre and post injection with p-value = 0.651 and 0.584 respectively. Also the table shows that there was no statistically significant increase in IOP post injection than pre injection in group B with p-value = 0.200.

Table (27): Comparison between group A and group C regarding IOP pre and post injection

		Group A	Group C	Test value	P-value	Sig.
IOP pre	Mean±SD Range	14.75 ± 1.62 13 – 17	14.28 ± 1.93 11 – 17	0.933	0.356	NS
IOP post	Mean±SD Range	14.75 ± 1.62 13 – 17	19.48 ± 2.62 14 – 24	7.678	<0.001	HS
Paired t-test	T		7.781			
	p-value		<0.001			

The previous table shows that there was no statistically significant difference found between group A and group C regarding IOP pre injection with p-value = 0.356 while there was highly statistically significant increase in IOP in group C than group A post injection with p-value < 0.001. Also the table shows that there was highly statistically significant increase in IOP post injection than pre injection in group C with p-value < 0.001.

Table (28): Comparison between group B and group C regarding IOP pre and post injection

		Group B	Group C	Test value	P-value	Sig.
IOP pre	Mean±SD Range	14.52 ± 1.94 12 – 18	14.28 ± 1.93 11 – 17	0.287	0.625	NS
IOP post	Mean±SD Range	14.84 ± 1.82 12 – 19	19.48 ± 2.62 14 – 24	7.282	<0.001	HS

The previous table shows that there was no statistically significant difference found between group B and group C regarding IOP pre injection with p-value = 0.625 while there was highly statistically significant increase in IOP in group C than group B post injection with p-value < 0.001.

Table (29): Comparison between group A and group B regarding central retinal thickness pre and post injection

Central retinal thickness		Group A	Group B	Test value	P-value	Sig.
Pre	Mean±SD Range	214.52 ± 12.70 190 – 240	375.56 ± 29.86 303 – 412	24.819	<0.001	HS
Post	Mean±SD Range	214.52 ± 12.70 190 – 240	329.92 ± 42.61 265 –405	12.978	<0.001	HS
Paired t-test	t p-value		5.209 <0.001			

The previous table shows that there was statistically significant difference found between group A and B pre and post injection with p-value <0.001 and <0.001 respectively. Also the table shows that there was statistically significant decrease in central retinal thickness in group B post injection than pre injection with p-value = < 0.001.

Table (30): Comparison between group A and group C regarding central retinal thickness pre and post injection

Central retinal thickness		Group A	Group C	Test value	P-value	Sig.
Pre	Mean±SD Range	214.52 ± 12.70 190 – 240	398.92 ± 38.61 328 – 450	22.687	<0.001	HS
Post	Mean±SD Range	214.52 ± 12.70 190 – 240	331.68 ± 41.54 282 – 421	13.486	<0.001	HS
Paired t-test	t p-value		11.456 <0.001			

The previous table shows that there was statistically significant difference found between group A and C pre and post injection with p-value <0.001 and <0.001 respectively. Also the table shows that there was statistically significant decrease in central retinal thickness in group C post injection than pre injection with p-value = < 0.001.

Table (31): Comparison between group B and group C regarding central retinal thickness pre and post injection

Central retinal thickness		Group B	Group C	Test value	P-value	Sig.
Pre	Mean±SD Range	375.56 ± 29.86 303 – 412	398.92 ± 38.61 328 – 450	2.393	0.021	S
Post	Mean±SD Range	329.92 ± 42.61 265 –405	331.68 ± 41.54 282 – 421	0.148	0.883	NS
Paired t-test	t p-value	5.209 <0.001	11.456 <0.001			

The previous table shows that there was statistically significant difference found between group B and C pre injection with p-value = 0.021 while no statistically significant difference between them regarding central retinal thickness post injection with p-value = 0.883.

Table (32): Comparison between the studied groups regarding visual acuity pre and post injection

Visual acuity		Group B	Group C	Test value	P-value	Sig.
Pre	Mean±SD Range	0.07 ± 0.06 0.02 – 0.2	0.10 ± 0.05 0.05 – 0.2	1.652	0.105	NS
Post	Mean±SD Range	0.41 ± 0.08 0.16 – 0.51	0.26 ± 0.11 0.1 – 0.4	5.407	<0.001	HS
Paired t-test	t p-value	5.209 <0.001 (HS)	11.456 <0.001 (HS)			

The previous table shows that there was no statistically significant difference found between the studied groups regarding visual acuity pre injection with p-value = 0.105. Also the table shows that there was statistically significant difference found between group B and C regarding visual acuity post injection with p-value < 0.001. The table also shows that there was highly statistically significant increase in visual acuity post injection than pre injection in group B and C with p-value < 0.001 and <0.001 respectively.

4. Discussion

Diabetic retinopathy (DR) is one of the most important causes of blindness worldwide and is the complication most feared by people with diabetes mellitus (DM). [10] DR is classically thought to result from microvascular changes in the retina, with microaneurysms—a result of ischemia due to capillary occlusion and nonperfusion—widely considered to be the first clinical sign of DR, [11] and pericyte loss considered as the earliest detectable histologic microvascular changes from diabetes in the retina. [12] Traditionally, retinal microvasculopathy has been seen as the pivotal initiating event, [13] followed by secondary inner retinal degeneration, termed retinal diabetic neuropathy (DRN). [14] Patients with DR may be asymptomatic, even in late stages of the disease, so early detection of the signs of DR is critical to limit visual loss from DR, especially now that numerous treatment options—laser, anti-vascular endothelial growth factor agents, and steroids [15] are available.

Disturbance of retinal capillary blood flow is a feature of many ocular diseases, including diabetic retinopathy and glaucoma. A number of non-invasive instruments have been designed to measure retinal haemodynamics.

In our present study we used scanning laser doppler flowmetry to measure retinal capillary blood flow, volume and velocity. This system is a well-established non-invasive technique which combines confocal laser scanning techniques and Laser Doppler Flowmetry. This method of measurement is influenced by ocular misalignment, camera distance from the patient eye, measurement variability within the brightest and the dimmest part of the scanned image which reflects the effect of the cardiac cycle [16]. To overcome problems in measurement variability, patients in the current study were instructed to keep their head stationary until the end of the measurement sequence and the camera was fixed at a predetermined distance from the eyes of all patients. Measurements were also taken from the brightest band in the area of interest from the better of two images and the difference in blood flow, volume and velocity

between the two eyes of each patient was calculated at same time.

In our present study the scanning laser Doppler retinal flowmeter was used to measure the retinal blood flow, volume and velocity of 25 eyes of normal individuals, 25 eyes of diabetic patients injected with ranibizumab and 25 eyes of diabetic patients injected with triamicinolone acetonide.

Hudson et al. [17] reported that temporal macular capillary blood flow was found to be significantly lower than that of age matched non-diabetic subjects and nasal-temporal asymmetry of macular capillary blood flow was significantly higher. Interestingly, temporal macular capillary blood flow was not significantly different between the patients with and without DMO and capillary leakage within the scan area whereas nasal-temporal asymmetry of macular capillary blood flow was significantly higher for the patients with DMO and capillary leakage within the scan area. For the five patients exhibiting DMO within the scanned area, the lower value of macular capillary blood flow was always to the side of the fovea exhibiting DMO and capillary leakage. Macular capillary blood flow showed substantial inter-individual variation both for the non-diabetic subject group and for the clinically significant DMO group; the standard deviation of macular capillary blood flow was approximately 50% of the group mean. The influence of the foveal avascular zone (FAZ), which is devoid of retinal capillaries, was probably reflected by the trend for group mean foveal macular capillary blood flow to be lower than either temporal or nasal blood flow. [17]

In a cross sectional study, Cuypers and co-workers (2000) found that retinal capillary blood flow measured by SLDF was associated with the level of diabetic retinopathy in the perifoveal macula area and approximately 7° nasal to the disc. Blood flow was reduced for patients with proliferative retinopathy in comparison with patients with pre-proliferative, or non-proliferative, retinopathy. Interestingly, blood flow was found to be reduced in patients with exudative maculopathy in comparison with patients with no, pre-proliferative, or proliferative, diabetic retinopathy. [18]

Rawji and Flanagan (2001) recently reported the magnitude of intraocular asymmetry of SLDF derived retinal blood flow parameters in clinically normal volunteers. As anticipated, capillary perfusion was found to increase with eccentricity from the fovea and no significant intraocular asymmetry was observed. [19]

Kern (1995) found that the relevance of the finding that temporal macular capillary blood flow was found to be significantly lower in patients with clinically significant DMO than that of age matched non-diabetic subjects has to be considered alongside the substantial inter-individual variation of the SLDF technique. In addition, there was a trend for the foveal and nasal capillary blood flow values to be lower but this did not reach statistical significance. Despite previous reports of a non-uniform distribution of diabetic vascular lesions, [20,21] the significantly lower temporal macular capillary blood flow can most likely be attributed to the lower inter-individual variability of the SLDF data at the temporal measurement site rather than any particular anatomical or pathophysiological influence.

Tsang and co-workers (1990) [22] found artefactual high SLDF blood flow values to occur as a result of reduced brightness. However, the retina exhibits a localized reduction of reflectance intensity in areas of DMO. [23] If the "brightness artefact" impacted upon the results of this study, macular capillary blood flow would be relatively higher in areas of DMO. Importantly, the finding that macular capillary blood flow was lower in areas of FFA leakage provides evidence of either a localized, rather than global, regulatory mechanism controlling macular capillary blood flow in patients with clinically significant DMO, or a local breakdown of this regulatory mechanism. The finding of a reduced blood flow in areas of FFA leakage is in broad agreement with the studies of Cuypers and co-workers (2000[24].

Some studies in the literature had shown that systemic, subtenon and topical steroids may cause changes in ocular blood flow [25-26]. It is not known how the ocular blood flow is affected by IVTA, however some studies have shown that the distal arterial obstruction increases the PSV of the arteries that supplies the eye [27-28]. TA may affect the ocular blood flow by its vasoconstructive effect on the peripheral arterial resistance [29-30]. Another mechanism may be due to the pass of TA in the retrobulbar area. Injected IVTA should pass through the sclera to the retrobulbar area to have an effect in the retrobulbar area [31-32].

Cekiç et al (2007) [33] reported that the EDV of PCA of the injected eyes decreased at the end of the first month and returned to normal values at the end of the third month after the IVTA injection.

It has been reported that before development of macular edema in diabetic retinopathy, diameter-response anomalies and loss of vascular tone were observed in retinal arterioles [34]. Also it has been reported that DME decreased after focal laser treatment, however the diameter response in the arterioles supplying this area did not change [35,36]. This information may explain why the RI was not affected after IVTA injection.

In our current study we found that the retinal capillary blood flow decrease in the two groups (Group B and Group C) than the normal individual in group A significantly. In Group B there was statistically significant decrease in retinal blood flow post injection than pre injection with p-value <0.001 in the upper arcade and <0.001 in the lower arcade while in Group C there was statistically significant decrease in retinal blood flow post injection than pre injection with p-value <0.001 in the upper arcade and lower arcade. While there is no statistically significant difference between both groups B and C following intravitreal injection of ranibizumab and triamcinolone acetonide as regards the retinal capillary blood flow.

In the study done by Yasin et al. (2014), shows that 4 mg/0.1 mL of TA has the increased PSV of OA and decreased the PSV CRA in the DME patients. The difference is very close to 0.05 significance level and increased number of the subjects may change the results. It has no effect on the ocular blood flow values of the CRVO and CNVM patients [37].

CDI and laser Doppler flowmetry have revealed that the velocity of blood flow in the ophthalmic artery and choroidal blood flow both decrease in eyes with DR [38,39]. In study done by Fumihiko et al. (2014), they believe that retinal autoregulation may be impaired in patients with DR and, furthermore, that the additional decrease in retinal circulation caused by IVB leads to an acceleration of the original chronic ischemia in eyes with DR, explaining the appearance of macular ischemia after IVB in patients with underlying diseases such as diabetes. Caution is therefore indicated when administering anti-VEGF antibodies, including bevacizumab, to DR patients. [40]

Arend and co-workers (1995) have previously reported that capillary blood velocity was significantly reduced in diabetic patients with cystoid macular oedema compared to non-diabetic subjects. This study also found that there was little difference in capillary blood velocity between diabetic patients with and without cystoid macular edema. [41]

In our present study there was a significant decrease in the velocity of the retinal blood flow in the upper and lower arcade post injection than pre injection in both Group B and Group C with p-value <0.001 in both groups while there was no significant difference found between group B and group C regarding the velocity of blood flow pre and post injection with p-value 0.136 and 0.647 respectively.

Sonja et al. (2018) [42], found the overall treatment response assessed as a change in BCVA and CRT are in accordance with previous studies, which have proven the clinical efficacy of ranibizumab and triamcinolone for DME therapy [43,44]. Consistent with the data from Protocol I of the DRCR, the net initial gain in visual acuity in patients treated with triamcinolone was lost within the second half of the year [45]. Even though a double dose of triamcinolone is used (8 mg instead of 4 mg in Protocol I) and the minimum treatment interval was shorter (12 instead of 16 weeks), the initial effect on DME resolution in the triamcinolone arm did not prevail as long as expected. After 3 months, patients treated with ranibizumab had a significantly thinner CRT than those treated with triamcinolone. Similar peaks of edema recurrence were seen in Protocol I at 16, 32 and 48 weeks after initial triamcinolone injection accordingly. Hence, reinjection of triamcinolone should be considered earlier in some patients, depending on morphologic dynamics, to avoid under-treatment.

Overall, CRT was similar in the treatment arms at baseline and at month 12, but BCVA was significantly better in the ranibizumab group after 12 months. A decrease in BCVA after 6 months of triamcinolone treatment is consistent with data published in a previous paper, where the loss in BCVA was attributed to cataract formation because pseudophakic patients treated with triamcinolone and those treated with ranibizumab had similar visual acuity results [46]. As cataracts were not to be operated during the study period, loss of BCVA in the triamcinolone-treated eyes might have been related to cataract formation.

In the study done by Berger et al. (2015), the participants received IVR injections alone during the 12-month follow-up. The major rescue protocol is macular photocoagulation and might reduce the frequency of IVR treatment. [47] Other publications have reported fewer injections of anti-VEGF drugs combined with triamcinolone, despite no differences in the visual outcomes. [48] Although there were no differences in the treatment frequency between eyes with and without VMT in the current study, most clinicians believe that VMT often prevents anti-VEGF treatment from achieving complete resolution of DME. [49] Other publications have reported the efficacy of IVR injections in vitrectomized eyes, which suggests that vitrectomy followed by anti-VEGF therapy might be a possible alternative strategy and reduce the treatment frequency in eyes with both DME and VMT. [50]

Wykoff et al. (2016), found that the association between the retinal thickness and treatment frequency with IVR injections suggests that the magnitude of vascular hyperpermeability might be related to the need for more frequent injections and might be consistent with the relationship between fluorescein leakage and the number of injections in a recent publication. [51]

Bressler et al. (2016), show that ranibizumab is superior to laser photocoagulation for the treatment of

DME. Ranibizumab has thus emerged as an excellent first-line therapy for DME, either as monotherapy or in combination with laser photocoagulation of the macula. Initial intensive therapy (monthly injections) appear to produce the best short-term and long-term results. Following initial resolution of the macular edema, physicians have the discretion to continue monthly therapy, treat recurrent edema as needed, or pursue a treat-and-extend strategy. Fortunately, excellent long-term results have been reported with each strategy. [52]

An Lai et al. (2017), found that Ranibizumab has been proven to be a safe treatment for providing visual improvement, reduced risk of DR progression and resolution of macular edema, with effects being detectable as early as one week after the initial IVR. After three monthly loadings of IVR in the present study, the mean logMAR of BCVA decreased from 0.81 to 0.62, and the mean CST decreased from 401 μm to 276 μm at month 3. [53]

According to the results of the study done by Sophie et al. (2015), patients with younger age and poorer baseline BCVA tended to have better visual improvement after three monthly loadings of IVR for DME. These results are compatible with the long-term outcomes of "DRCR.net" and the RISE and RIDE studies, which showed that younger-aged patients and poorer baseline BCVA were associated with better long-term visual improvement. [54] This can be explained by the "ceiling effect", since those with a poorer baseline BCVA have more room for visual improvement. This ceiling effect can also explain why thicker baseline CST tends to have more of a reduction in CST in the present study as well as the "DRCR.net" study. [54]

Shinri Sato et al. (2017), reported the therapeutic effects of monotherapy with an anti-VEGF drug, ranibizumab, without rescue treatment for DME in various types of patients encountered in daily clinical practice. Although the mean BCVA remained unchanged, the mean CRT was significantly reduced by IVR in patients with DME included in the study. Those who had a poorer BCVA or greater CRT at baseline, and who had already undergone PRP before the initial IVR injection due to the progress of diabetic retinopathy, achieved greater improvements at 12 months. A CRT improvement of more than 100 μm was associated with a greater CRT at baseline. However, BCVA and CRT at 12 months were positively correlated with baseline measurements, and better BCVA and CRT values were achieved at 12 months in patients who had better BCVA and a milder CRT increase at baseline. [55]

In the study done by Masahiro (2018), a single intravitreal injection of ranibizumab reduced the retinal thickness and improved the BCVA in eyes with DME in both the no-PRP group and PRP treated group there were no significant changes in the choroidal thickness, total choroidal area, and choroid blood flow in eyes with DME during the follow up period after intravitreal injection of ranibizumab. [56]

In our current study there was statistically significant difference found between the studied groups regarding visual acuity pre and post injection with p-value <0.001 and <0.001 respectively while the visual acuity improves in both groups post injection with a highly statistically significant difference with p-value <0.001. As regards the central retinal thickness, our current study shows that there was no statistically significant difference between both groups B and C pre and post injection while there was a statistically significant decrease in central retinal thickness in both groups post injection with p-value <0.001.

Intravitreal injections of anti-VEGF agents can resolve the macular edema, subretinal fluid, and neovascularization very rapidly. [57, 58] Previous studies using Doppler imaging on eyes with AMD and DME showed that the retrobulbar circulation decreased after a bevacizumab injection. [59, 60]

A LSFG study also found a decrease in the ocular circulation after an intravitreal anti-VEGF agent injection in the treated eye at different stages of diabetic retinopathy (DR), [61] and MBR was reported to be significantly correlated with the foveal thickness. [62,63] These findings indicate that anti-VEGF agents affect the ocular circulation associated with a reduction of the CMT.

Anti-VEGF agents are effective not only for ME resolution but also on vascular contraction. This fact indicates that the vascular autoregulation works well for eyes with a resolution of the ME. There is a possibility that these changes are related to the ME regression and the integrity of the blood vessels. Although an intravitreal injection of anti-VEGF agents affected the blood flow strongly, it only affected the treated eyes and not the fellow untreated eyes. Other reports did not mention the circulation of the untreated eyes. In study done by Masahiko et al. (2017) [65] they found that dMBR decreased for treated eyes and slightly increased for untreated eyes. The reason for the increase in the untreated eye is not clear, but because this increase was not significant from comparison of raw MBR value, so this change can be ignored. [64, 65]

Degenring et al. (2004) found that after intravitreal triamcinolone acetonide injections, the systemic concentrations of corticosteroid were not altered, which suggests that agents that are given intravitreally generally do not enter the systemic circulation in effective concentrations [66]. However, the pharmacokinetics of drugs differ, and it is difficult

to compare them. Anti-VEGF agents are also approved for DME, although it is well known that vascular infarction is a major compilation for diabetic patients, and painless myocardial infarction or micro-cerebral infarction is also higher in diabetic patients [67, 68]. There have been at least two studies on the systemic risks of intravitreal anti-VEGF agents for a high-risk group and a diabetes group [69, 70].

In our current study we found no obvious systemic changes including any thromboembolism event during the observation so we need to pay special attention to diabetic patients.

Study done by Mojica et al. (2008) investigating ranibizumab, confirmed a spike in IOP shortly after injection but continued to monitor the patients. Despite an increased IOP at 30 min., the study found no significant difference between pre- and post-injection IOP at follow up period thus ranimizumab has no effect on IOP. [71]

In the study done by Ashiyana et al., (2016) the average pre-injection IOP was 15.7 mmHg and after one year was 15.2 mmHg after receiving three injections of ranibizumab so there is no increase in IOP following intravitreal ranibizumab injection in DME patients regardless how many injections they receive. [72]

In the study done by Alice et al. (2014) they did not find an increase in IOP in frequently ranibizumab treated eyes in DME [73].

As has been reported in previous studies, intravitreal injection of triamcinolone acetonide is associated with an increased risk of IOP elevation. In the study of Audren et al. (2006) seven out of 15 patients (47%) in the triamcinolone group had at least one IOP measurement >25 mmHg compared with only one (10%) in the ranibizumab group, all patients responded well to IOP-lowering medication. [74]

The IOP rise after intravitreal injection of triamicinolone is thought to be due to activation of glucocorticoid receptors in the trabecular meshwork which cause biochemical and ultrastructural changes in the meshwork resulting in greater resistance in the aqueous outflow. [75] In the study done by O'Day et al. (2014), they found an increased IOP in the first 6 month after a single injection of triamicinolone for DME. [76]

In the study done by Jonas et al. (2003), the IOP after intravitreal injection of triamcinolone acetonide increased significantly ($p<0.001$) from 15.43 mmHg preoperatively to 23.38 mmHg postoperatively and this IOP is normalized by topical medication and return to normal about 6 months after injection. [77]

In our current study there was no statistically significant difference between group A and group B as regards IOP pre injection and post injection with p-value 0.651 and 0.584 respectively also there was no statistically significant increase in IOP post injection than pre injection in group B with p-value 0.200. Also there was no statistically significant difference between group A and group C regarding IOP pre injection with p-value 0.356. While there was highly statistically significant increase in IOP in group C than group A post injection with p-value <0.001. There was highly statistically significant increase in IOP in group C than in group B post injection with p-value <0.001.

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