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Ophthalmology

KEY WORDS: Diabetic Macular Edema, Ranibizumab, Laser Photocoagulation, Visual Acuity, Central Macular Thickness.

COMPARATIVE EFFICACY OF INTRAVITREAL RANIBIZUMAB MONOTHERAPY WITH COMBINED INTRAVITREAL RANIBIZUMAB AND LASER PHOTOCOAGULATION THERAPY IN THE MANAGEMENT OF DIABETIC MACULAR EDEMA.

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ABSTRACT

Background: In this study, best corrected visual acuity and macular thickness were compared before and after intravitreal injection Ranibizumab monotherapy and combined therapy of injection Ranibizumab and laser, in patients with macular oedema ≥350µm measured with spectral domain OCT. **Objective:** Our specific objective is to identify the best treatment option in NPDR with macular thickness ≥350µm in Type 2 diabetic retinopathy patients depending upon the anatomical and functional outcome in each group. **Method:** Sixty (60) newly diagnosed eyes of NPDR with macular oedema (30 patients in each group) attending retina research clinic of Regional Institute of Ophthalmology, Kolkata were included in this study. **Result:** This parallel group comparison trial has shown that a combination of 3 consecutive monthly doses of intravitreal ranibizumab followed by modified grid laser therapy 7-10 days latter is more effective in reducing central macular thickness in comparison to only multiple injections of ranibizumab, but there is no difference in the final best corrected visual acuity attained in between the two groups. **Conclusion:** Laser photocoagulation along with anti-vascular endothelial growth factor agent is the stronger weapon to fight against blindness in diabetic macular edema.

Introduction:

Diabetic retinopathy (DR), the leading cause of visual disability in diabetics, is an important complication of diabetes mellitus (DM) [1-3]. The prevalence of DR in India ranges from 17.6% to 28.2% [4-6]. This overwhelming burden needs an effective and well defined treatment strategy. Diabetic macular oedema (DME), a complication of diabetic retinopathy, is an important cause of blindness among the working age. The prevalence of DME is likely to increase with more people suffering from diabetes[7]. Increasing DME has significant implications for patients, healthcare providers and wider society in general.

The pathogenesis of DME is multifactorial. It is predominantly due to a generalized breakdown of the inner blood retinal barrier, leading to accumulation of fluid and plasma constituents, such as lipoproteins, within the intraretinal layers of macula [8-9]. Factors such as duration of diabetes, insulin dependence, glycosylated haemoglobin levels, proteinuria and hypertension have all been implicated in the development of DME. VEGF has been identified as one of many growth factors that breakdown the blood retinal layer causing increased retinal permeability by affecting the endothelial tight junctions [10]. The normal human retina contains VEGF; however the levels are significantly elevated in eyes with DME [11]. The pathogenesis of retinal vascular permeability has also been attributed to inflammation, particularly via leucostasis within the retinal capillaries. The attraction and adhesion of leucocytes to the vascular wall, in the setting of diabetes, may be due to an increased expression of leucocyte adhesion molecules such as retinal endothelial cell intercellular adhesion molecule 1 and CD18[12-13]. Therefore attenuation of the effects of VEGF and a reduction in inflammation may reduce macular edema associated with diabetes.

Laser photocoagulation has been the mainstay of treatment for DME. Its mechanism is the probable rejuvenation of retinal pigment epithelial cells or improvement of outer retinal oxygenation. The landmark Diabetic Retinopathy Study [14] and the Early Treatment Diabetic Retinopathy Study (ETDRS)[15-16] demonstrated its clinical effectiveness. However, although laser photocoagulation was clearly effective in preserving vision, it was less successful in restoring it, once lost. In EDTRS, although laser was shown to reduce the risk of moderate visual loss (a loss of three ETDRS lines) by 50%, visual acuity improved in only 3% of patients [16]. However, in some recent trials, laser has improved the proportion of patients with more than or equal to 10 letters by 7–31% [17-20]. In addition, laser is not without side effects. Foveal burns, visual field defects, retinal fibrosis and laser scars have been known to occur [21]. Over the following decade it became apparent that certain patients suffered severe visual loss despite aggressive treatment [22].

More recently, anti-vascular endothelial growth factor (anti-VEGF) injections have become the standard of care for eyes with DME and vision impairment [23]. A number of studies have shown that anti-VEGF injections are effective in reducing central macular thickness (CMT) and improving best-corrected visual acuity (BCVA) [24-25]. However, anti-VEGF agents are not able to treat macular hypoxia, which is the main cause of DME [24], and thus the effects of anti-VEGF agents are transient. In addition, the duration of action of anti-VEGF agents is limited, as shown by the short half-life of bevacizumab and ranibizumab in eyes, both anti-VEGF agents, of 9.8 days and 2.75 days, respectively, resulting in the recurrence of macular edema in eyes treated with anti-VEGF agents within a few weeks [25-28].

Consequently, the combination of laser macular photocoagulation (MPC) and anti-VEGF injections may be an effective therapy to prolong the effectiveness of anti-VEGF agents and contribute to a better result by reducing macular hypoxia. Also, MPC becomes easier due to a reduction of macular edema by anti-VEGF injections. A number of recent studies have indicated that treatment with anti-VEGF agents in combination with MPC is superior to therapy with an anti-VEGF agent alone in terms of reducing CMT and improving BCVA in patients with DME [26-30]. However, other studies have reported that there was no significant difference in CMT reduction and BCVA improvement between DME patients

given anti-VEGF agents in combination with MPC and those given anti-VEGF agents alone [30-31].

In our present study, we have compared the efficacy of intravitreal (anti-VEGF) injection Ranibizumab versus a combination therapy of injection Ranibizumab and laser in the treatment of DME in Type 2 non-proliferative diabetic retinopathy (NPDR) patients, so as to find out the best therapy for DME in a developing country like India, where repeated intravitreal injections of ranibizumab will certainly create a financial constrain.

A Pilot studies with intravitreal Ranibizumab have also shown decrease in mean retinal thickness and improved vision in patients with DME. In three well designed, phase II or III trials (RESOLVE [32], RIDE and RISE [33], READ 2[34]) 1-2 year treatments with Ranibizumab were found to be more effective than sham or focal/grid laser therapy in improving best corrected visual acuity(BCVA) and reducing macular thickness in patients with visual impairment associated with DME. Along with these studies, two other well designed phase III trials (RESTORE [35] and DRCR.net[36]) showed that one year of treatment with Ranibizumab as an adjunct to laser therapy showed improvement at the first follow-up visits in these studies, and were associated with gains in vision related quality of life. Repeated intravitreal injections of this drug was used for this study.

MATERIALS AND METHODSA:

STUDY AREA

This study was done at the Regional Institute of Ophthalmology, Medical College and Hospital, Kolkata. The subjects belong to various districts of West Bengal.

STUDY POPULATION

Patients with Type 2 diabetes mellitus, who had diminution of vision only due to Non- proliferative diabetic retinopathy (NPDR) with diabetic macular oedema (DME) and not due to ischemic diabetic maculopathy, were randomly selected from outpatient department of Regional Institute of Ophthalmology, Kolkata. Further, patients with macular oedema≥350µm, found within central (1 mm diameter) subfield area in SD-OCT were involved in this study. Evaluation and management of all the cases were performed at the Retina Research Clinic of the Regional Institute of Ophthalmology, Kolkata.

INCLUSION CRITERIA:

1. Patients having non -ischemic, non -proliferative diabetic retinopathy with macular oedema (≥350 um), found within central (1 mm diameter) subfield area in SD-OCT, all diagnosed and well controlled Type 2 Diabetes Mellitus, only newly diagnosed cases of NPDR with DME, not received any form of treatment (focal/grid laser or injection Ranibizumab/any form of intravitreal anti VEGF or intravitreal triamcinolone injection /any form of intravitreal steroid injection) in the past, including posterior sub Tenon steroid injection.

EXCLUSION CRITERIA: Type 1 diabetes mellitus, Patients suffering from uncontrolled hypertension, thyroid eye disease, pregnancy, uncontrolled hypercholesteremia, anemia, renal disease or having some other systemic disease, which may affect the retinal thickness as judged by the treating ophthalmologist.

STUDY DESIGN:

An open label, randomized, parallel group, comparative trial.

SAMPLE SIZE:

60 newly diagnosed eyes of patients of NPDR with macular oedema ≥350µm. A sample size of 30 in each of the two groups was selected.

SAMPLE DESIGN:

A computer- generated randomization schedule was followed.

STUDY PARAMETERS:

1. The main outcome measures studied was the change in macular thickness within central subfield (1 mm area) in SD-OCT, assessed in 3 follow-up visits
in each group of Injection ranibizumab monotherapy and combined Injection ranibizumab and laser group, as evaluated by SD-OCT.
2. Scores for ETDRS visual acuity were measured at every follow- up.
3. Intraocular pressure (IOP) was measured by Goldmann Applanation Tonometer at the start and at the end of the study and also recorded when felt necessary.
4. Dilated fundus examination by slit lamp biomicroscopy with +90 dioptres and peripheral retinal examination with indirect ophthalmoscopy was performed in both eyes at every follow up and also when felt necessary.
5. Fluorescein angiography was performed to rule out ischemic diabetic retinopathy and proliferative diabetic retinopathy at the start of the patient enrollment.

SD-OCT technique:

After appropriate pupillary dilatation using Phenylephrine 5% +tropicamide 0.8% eye drops, the patient was seated comfortably on a chair in front of the device, in a dimly lit room, asked to place their chin on the chin rest, and to look at the centre of the green target, and not at the moving light inside the imaging aperture. The OCT volume scan was performed on a 20×20 degree cube with 49 raster lines, each containing 1064 pixels, separated by 120 µ. The high acquisition speed of 40,000 A- scans/ second avoids artifacts from micro saccades and improves image definition.

STUDY TECHNIQUE:

This prospective, randomized clinical study was done on 60 eyes of patients having NPDR with macular oedema ≥350µm in the central subfield (central 1 mm area) as per SD-OCT. Patients enrolled for this study had to undergo tests like FBS, PPBS, Hba1c, urea, creatinine, complete blood count, lipid profile , blood pressure checkup and cardiological fitness. Then the patients had to undergo visual acuity tests (ETDRS chart), Slit lamp examination, dilated fundus examination with direct and indirect ophthalmoscope including slit-lamp biomicroscopy with +90D, applanation tonometry, fluorescein fundus angiography (FFA) and spectral domain OCT (macular thickness mapping). Patients were randomized either in the Injection ranibizumab monotherapy group or in the combined group of Injection ranibizumab with modified grid laser. Both groups received monthly consecutive 3 intravitreal injections of Ranibizumab 0.5 mg initially, followed by which one group received focal/grid laser photocoagulation within 7-10 days, after the 3rd injection, while another group was treated by repeated intravitreal administration of Injection Ranibizumab, when warranted until the end of 6 months.

PROCEDURE:

Intravitreal Ranibizumab injection: After taking written consent from the patient, six days prior to procedure patient was asked to instill eye drop Moxifloxacin (0.5%) 4 times/ day in the affected eye. On the day of procedure, the affected eye was dilated with combined topical preparation of Tropicamide (0.8%) and Phenylephrine hydrochloride (5%) eye drop. Then the eye is anesthesized with 0.5% topical proparacaine hydrochloride 2-3 minutes prior to injection. The eyelid and skin was cleaned with 10% Povidone- iodine and one drop of 5% Povidone –iodine was kept in the conjunctival sac for 3 minutes and then thoroughly washed with balanced salt solution and properly draped. Then 0.5 ml (0.5mg) of injection Ranibizumab will be loaded into an insulin syringe (30 gauges). After separating the lids with a

wire speculum, 0.05 ml of Ranibizumab will be injected into the vitreous cavity through the inferior- temporal pars plana, 3.5 – 4 mm away from the limbus. Tamponade was applied at the site of injection with a swab stick for 2-3 mins. Topical antibiotic drop will be instilled after the injection. Patient was asked to remove the eyepad after 2 hours and also asked to instill eye drop Moxifloxacin (0.5%) 4 times/ day and anti-glaucoma medication for 7 days. The intravitreal injection was given for 3 consecutive months initially, and if macular oedema persisted as assessed by SD-OCT, it was followed up by further additional doses of injection Ranibizumab until the 6th month, in one group of patients. All the injections were given by a single surgeon. Intra-ocular pressure checkup and indirect ophthalmoscopy with scleral depression was performed after each intravitreal injection.

Combined therapy: Here, patients will be given intravitreal Ranibizumab injection first for 3 consecutive months. Then if macular edema still persists, as assessed by SD-OCT, the 3rd dose of intravitreal ranibizumab will be followed by a modified grid laser after 7-10 days of the 3rd injection Ranibizumab, and the final follow up visit will be done at 6th month.

All patients in both procedures were followed up on all the first post-operative days, and then 1 week after the 1st Injection Ranibizumab (First follow-up), 1 week after 2nd Injection Ranibizumab (Second follow-up) and then at 6 months after 3rd Injection Ranibizumab+ grid laser at 7-10 days or after 3rd Injection Modified grid laser technique employs primarily grid treatment to areas of diffuse leakage with occasional focal treatment of focal leakage located either within or outside the areas of diffuse edema. Laser machine is adjusted with retinal spot size of 50-100 µm, duration of 0.1 seconds with light intensity to obtain gentle whitening. Laser spots are applied one burn width apart staying 500µm away from the foveal centre and the disc margin to up to 3000 µm from the macular centre excluding the area of papillomacular bundles. Foveal avascular zone is fastidiously avoided to prevent central scotomas. Modified grid laser photocoagulation is performed in 2-3 rows.

Ranibizumab+ additional monthly Injections Ranibizumab, if needed until 6 months (3rd Follow up). Best corrected visual acuity (BCVA) was assessed with ETDRS chart at each visit and macular edema was evaluated with Spectral domain Optical Coherence Tomography (Central subfield) at the end of 1st ,2nd and 3rd follow-ups. Any complications following the procedure were managed accordingly.

Modified grid laser technique employs primarily grid treatment to areas of diffuse leakage with occasional focal treatment of focal leakage located either within or outside the areas of diffuse edema. Laser machine is adjusted with retinal spot size of 50-100 µm, duration of 0.1 seconds with light intensity to obtain gentle whitening. Laser spots are applied one burn width apart staying 500µm away from the foveal centre and the disc margin to upto 3000 µm from the macular centre excluding the area of papillomacular bundles. Foveal avascular zone is fastidiously avoided to prevent central scotomas. Modified grid laser photocoagulation is performed in 2-3 rows.

RESULTS AND ANALYSIS

Statistical Analysis:

Statistical Analysis was done by using descriptive and inferential statistics using Chi-square test, one-way ANOVA, repeated measures ANOVA and unpaired student's t- test. The software used in the analysis were IBM SPSS Statistics and $p<0.05$ is considered as level of significance ($p<0.05$).

Table1. Age wise distribution of patients in two groups

AGE Group (yrs)	RANIBIZUMAB (n=30)	RANIBIZUMAB+ LASER(n=30)	X^2 value
41-50	10	9	2.219 p=0.53,NS
51-60	13	11	
61-70	6	10	
>70	1	0	
TOTAL	30	30	
Mean	54.83	57.1	
SD	8.03	7.81	

The above table shows that in both the two groups, maximum cases were in the age group of 51-60 yrs. Age distribution in the two groups was statistically non-significant(p=0.53)

Table 2: Gender wise distribution of patients in two groups

GENDER	RANIBIZUMAB (n=30)	RANIBIZUMAB+ LASER(n=30)	X^2 value
Male	19	23	1.27 p=0.26,NS
Female	11	7	
TOTAL	30	30	

The above table shows that gender wise distribution in the two groups was statistically non- significant (p= 0.26)

Table 3.1: Descriptive Statistics

Groups	Mean CMT	Std.Deviation	Std .Error	Minimum	Maximum
Ranibizumab	413.5	37.74	6.89	362	512
Ranibizumab+ Laser	416.4	41.51	7.58	355	520

Table 3.2: Unpaired student's t-test (CMT)

GROUPS	N	df	t-statistic	t-critical	p-value
Ranibizumab	30	57	-0.29	1.67	0.78, NS
Ranibizumab +Laser	30				

Baseline CMT measurements distribution in the two groups was statistically non-significant with a p-value= 0.78, as per the unpaired student's t-test.

Table 4: Comparison of BCVA in two groups at baseline

Table 4.1 Descriptive Statistics

Groups	Mean	Std. Deviation	Std. Error	Minimum	Maximum
Ranibizumab	1.03	0.1	0.02	0.78	1.17
Ranibizumab +Laser	0.99	0.12	0.02	0.78	1.17

Mean baseline BCVA measurements in the groups Ranibizumab and Ranibizumab+Laser are 1.03 LogMAR and 0.99 LogMAR respectively.

Table4.2 Unpaired student's t-test (BCVA)

GROUPS	N	df		t-critical	p-value
Ranibizumab	30	29	1.01	2.05	0.32, NS
Ranibizumab +Laser	30				

Baseline BCVA measurements in the two groups was statistically non- significant with p-value 0.32 as per unpaired student's t-test.

GROUP 1: INJECTION RANIBIZUMAB

Table 5: Comparison of CMT in group Ranibizumab at 1st follow up, 2nd follow up and 3rd follow up.

Table 5.1: Descriptive Statistics

	Mean	N	Std. Deviation	Std. Error
Baseline	413.5	30	37.74	6.89
1st follow up	357.2	30	34.65	6.33

2nd follow up	320.9	30	27.09	4.95
3rd follow up	286.8	30	26.16	4.78

Mean CMT measurements in the group Ranibizumab at baseline, 1st follow up, 2nd follow up and 3rd follow up are 413.5, 357.2, 320.9 and 286.8 µm respectively.

Table 5.2 Repeated measures ANOVA

Source of Variation	Sum of squares	Df	Mean Square	F	P-value	F crit
Within groups	51023.57	29	1759.43	4.09	<0.0001,S	1.7
Between groups	74507.47	2	37253.73	86.67	<0.0001,S	3.2
Error	24929.87	58	429.83			
Total	150460.9	89				

The foveal thickness was found to be improved at 1st follow up, 2nd follow up and 3rd follow up after 3 consecutive monthly doses of Inj. Ranibizumab, with additional doses of Injection Ranibizumab, as required, until 6 months.

Table 6: Comparison of BCVA in group Ranibizumab at 1st follow up, 2nd follow up and 3rd follow up.

Table 6.1: Descriptive Statistics

	Mean	N	Std. Deviation	Std. Error
Baseline	1.03	30	0.1	0.02
1st follow up	0.91	30	0.15	0.03
2nd follow up	0.89	30	0.16	0.03
3rd follow up	0.88	30	0.15	0.03

Mean BCVA measurements in the group Ranibizumab at baseline, 1st follow up, 2nd follow up and 3rd follow up are 1.03 ±0.1 LogMAR, 0.91±0.15 LogMAR , 0.89±0.16 LogMAR and 0.88±0.15 LogMAR respectively.

Table 6.2 Repeated measures ANOVA

Source of Variation	Sum of Squares	Df	Mean Square	F	P-value	F crit
Within groups	1.85	29	0.06	23.15	0.0001,S	1.66
Between groups	0.02	2	0.01	3.39	0.04,S	3.16
Error	0.16	58	0.003			
Total	2.03	89				

Using the repeated measures ANOVA test, p value for patients receiving 3 doses of Inj. Ranibizumab initially followed by additional doses of Ranibizumab (as warranted), compared for BCVA at 1st follow up, 2nd follow up and 3rd follow up was statistically significant (p= 0.04). There was a significant improvement in BCVA in patients treated with Injection Ranibizumab over 6 months follow up.

GROUP 2: INJECTION RANIBIZUMAB+MODIFIED GRID LASER

Table 7: Comparison of CMT in group Ranibizumab+ Laser at 1st follow up, 2nd follow up and 3rd follow up.

7.1: Descriptive Statistics

	Mean	N	Std. Devation	Std. Error
Baseline	416.4	30	41.51	7.58
1st follow up	350.7	30	47.73	8.71
2nd follow up	296.6	30	29.63	5.41
3rd follow up	251.4	30	18.55	3.39

Mean CMT measurements in the group Ranibizumab+ Laser at baseline, 1st follow up, 2nd follow up and 3rd follow up are 416.4, 350.7, 296.6 and 251.4 µm respectively.

Table7.2 Repeated measures ANOVA

Source of Variation	Sum of Squares	df	Mean Square	F	P-value	F crit
Rows	43393.12	29	1496.31	1.49	0.10,NS	1.66
Columns	148399.76	2	74199.88	74.05	<0.0001,S	3.16
Error	58114.91	58	1001.98			
Total	249907.79	89				

The foveal thickness was found to be improved at 1st follow up, 2nd follow up and 3rd follow up after 3 consecutive monthly doses of Inj. Ranibizumab along with modified grid laser at 7-10 days, when followed up over 6 months.

Table 8: Comparison of BCVA in group Ranibizumab+ modified grid laser at 1st follow up, 2nd follow up and 3rd follow up.

Table 8.1: Descriptive Statistics

	Mean	N	Std. Devation	Std. Error
Baseline	0.99	30	0.12	0.02
1st follow up	0.91	30	0.15	0.03
2d follow up	0.87	30	0.17	0.03
3rd follow up	0.87	30	0.17	0.03

Mean BCVA measurements in the group Ranibizumab+Laser at baseline, 1st follow up, 2nd follow up and 3rd follow up are 0.99 ±0.12 LogMAR, 0.91±0.15 LogMAR , 0.87±0.17 LogMAR and 0.87±0.17 LogMAR respectively.

Table8.2 Repeated measures ANOVA

Source of Variation	Sum of Squares	df	Mean Squares	FF	P-value	crit
Rows	2.23	29	0.08	31.67	<0.0001	1.66
Columns	0.03	2	0.01	5.63	0.005,S	3.16
Error	0.14	58	0.00			
Total	2.40	89				

Using the repeated measures ANOVA test, p value for patients receiving 3 doses of Inj. Ranibizumab with modified grid laser compared for BCVA at 1st follow up, 2nd follow up and 3rd follow up was statistically significant (p= 0.005). There was thus significant improvement in BCVA in patients treated with Inj Ranibizumab with modified grid laser at 7-10 days, over 6 months follow up.

One way ANOVA(CMT)

Time Interval	Source of Variation	Sum of squares	df	Mean square	F	p-value
Baseline	Between groups	129.07	1	129.07	0.08	0.78, NS
	Within groups	91276.87	58	1573.74		
	Total	91405.93	59			
1st follow up						
	Between groups	633.75	1	633.75	0.36	0.55, NS
	Within groups	100877.2	58	1739.26		
	Total	101511	59			
2nd follow up						
	Between groups	2148.02	1	2148.02	3.09	0.08, NS
	Within groups	40342.17	58	695.55		
	Total	42490.18	59			

3rd follow up						
	Between groups	18762.02	1	18762.02	36.5	<0.0001, S
	Within groups	29836.57	58	514.42		
	Total	48598.58	59			

Mean CMT at the start of the treatment i.e. the baseline, at 1st follow up and at 2nd follow up was statistically insignificant in between and within the two groups but it became significant at 3rd follow up at 6 months(p=0.0001), using one-way ANOVA. Therefore, a significant difference in CMT was seen when modified laser was added to the three consecutive monthly doses of Injection Ranibizumab 7-10 days later, and followed up for 6 months.

Table 9: Comparison of BCVA in both groups at baseline, 1st follow up, 2nd follow up and 3rd follow up.

One way ANOVA (BCVA)

Time Interval	Source of Variation	Sum of squares	df	Mean square	F	p-value
Baseline	Between groups	0.024	1	0.024	1.9	0.2,NS
	Within groups	0.713	58	0.012		
	Total	0.737	59			
1st follow up						
	Between groups	0.0003	1	0.0003	0.02	0.9,NS
	Within groups	1.36	58	0.023		
	Total	1.36	59			
2nd follow up						
	Between groups	0.003	1	0.003	0.12	0.7,NS
	Within groups	1.6	58	0.027		
	Total	1.6	59			
3rd follow up						
	Between groups	0.0014	1	0.0014	0.06	0.8,NS
	Within groups	1.428	58	0.025		
	Total	1.429	59			

Mean BCVA at the start of the treatment i.e. the baseline, and at 1st follow up, 2nd follow up and 3rd follow up was statistically insignificant in between and within the two groups , using one way ANOVA. Therefore, no significant difference in BCVA was seen when modified laser was added to the three consecutive monthly doses of Injection Ranibizumab 7-10 days later, and followed up for 6 months.

Discussion:

This present study aimed to find the efficacy of 0.05 ml (0.5 mg) 3 doses intravitreal Ranibizumab therapy with required additional doses (if macular oedema persisted as seen on 3DOCT) against combined treatment of intravitreal 3 doses Ranibizumab and modified grid laser treatment in patients of NPDR with macular oedema ≥350µm in the central subfield(central 1 mm area) as per SD-OCT and on best corrected visual acuity (BCVA) as per ETDRS chart at 1st follow up, 2nd follow up and 3rd follow up , over a period of 6 months, and also to find the best treatment option amongst the two groups.

In this study, total 60 patients were taken (sample size 30 in each group) with age group of more than 40 years, 42:18 male: female ratio, and 29:31 right eye: left eye ratio. Maximum number of patients were 51-60 years of age and statistical analysis has shown that age distribution, gender distribution and side of eye involvement had no effect on type of treatment chosen with p=0.53, p=0.26 and p=0.80 respectively which is insignificant. In the groups Ranibizumab and Ranibizumab +modified grid laser, mean baseline central macular thickness(CMT) measurements was 413.5±6.89 µm and 416.4±7.58 µm respectively, and mean baseline BCVA measurements was 1.03±0.02 LogMAR and 0.99±0.02LogMAR respectively. There was no effect of baseline CMT and baseline BCVA measurements in the two groups with type of treatment chosen for baseline. The p value of the two groups baseline was statistically non-significant (p= 0.78 in baseline CMT and p= 0.32 in baseline BCVA) as per unpaired student's t -test (Unpaired student's t-test is used to determine whether there is any significant differences between the means of two independent groups).

GROUP 1: INJECTION RANIBIZUMAB:

In group 1, the baseline central macular thickness (CMT) was 413.5±6.89µm. At 1st follow up (following 1st Injection Ranibizumab), mean CMT decreased from baseline of 413.5±6.89µm to 357.2±34.65µm, a difference of 56.3µm (p<0.0001) was noted and this overall improvement continued at the 2nd and 3rd follow up. 2nd follow up was after the 2nd Injection Ranibizumab, and 3rd follow up was at 6 months after 3rd Injection Ranibizumab+ additional monthly doses if required. The requirement of additional monthly doses of Ranibizumab was decided on the persistence of macular edema on SD-OCT. Amongst the 30 patients, 9 patients (30%) required an additional 4th dose of injection Ranibizumab and 8 patients (27%) required an additional 5th dose of Inj. Ranibizumab. At 2nd and 3rd follow-ups, mean central macular thicknesses were further decreased to 320.9±27.09µm (difference of 92.6 µm with baseline) and 286.8±26.16µm (difference of126.7 µm with baseline) respectively with p value 0.0001 for both. Hence, there was statistically significant improvement at 1st follow up, 2nd follow up and 3rd follow up CMT over 6 months, with intravitreal injection of consecutive 3 doses monthly Ranibizumab (and additional doses, if required) when compared to baseline.

Mean BCVA measurements in the Ranibizumab group at baseline, 1st follow up, 2nd follow up and 3rd follow up were 1.03±0.1LogMAR ,0.91±0.15 LogMAR, 0.81±0.16 LogMAR and 0.88±0.15 LogMAR respectively. Using repeated measures ANOVA test, p value for patients receiving intravitreal Ranibizumab compared for BCVA at 1st follow up, 2nd follow up and 3rd follow up with the baseline and found to be statistically significant (p=0.04). Hence, there was significant improvement in BCVA in patients treated with intravitreal Ranibizumab at 1st follow up, 2nd follow up and 3rd follow up when compared with baseline BCVA.

The RIDE and RISE study[33] was a 3 year trial study comparing the effect of intravitreal Ranibizumab to sham injections in DME patients having a central foveal thickness of >275um. It concluded that intravitreal Ranibizumab sustainably improved vision, reduced the risk of further deterioration of vision and improved macular edema with low rates of ocular side effects.

GROUP 2: INJECTION RANIBIZUMAB+MODIFIED GRID LASER:

In group 2 (combined group) mean CMT measurements at baseline, 1st follow up, 2nd follow up and 3rd follow up were 416.4±41.51µm, 350.7±47.73µm, 296.6±29.63µm and 251.4±18.55 µm respectively. The foveal thickness improved over 6 months after combined treatment. At 1st follow up, mean CMT decreased from baseline of 416.4±41.51µm to 350.7±47.73µm, a difference of 65.7 µm (p=0.0001) and this

overall improvement continued at the 2nd and 3rd follow up over 6 months. The difference in CMT from the baseline at 2nd follow up was 119.8 μm and the difference in CMT from the baseline at 3rd follow up was 165 μ, with a p value of 0.0001 for both. So, there was statistically significant improvement at 1st follow up, 2nd follow up and 3rd follow up CMT with combined treatment when compared to baseline. In combined group, overall 30 patients of NPDR with CMT≥350μm received 3 consecutive monthly doses of inj. Ranibizumab followed by modified grid laser 7-10 days later and were evaluated for CMT by SD-OCT after the 1st injection Ranibizumab(1st follow up), 2nd Injection Ranibizumab(2nd follow up) and 3rd Inj. Ranibizumab+ modified grid laser done 7-10 days later and evaluated at month 6 (3rd follow up) respectively.

Mean BCVA measurements in the Ranibizumab+modified grid laser group at baseline, 1st follow up, 2nd follow up and 3rd follow up were 0.99±0.12 LogMAR ,0.91±0.15 LogMAR, 0.87±0.17 LogMAR and 0.87±0.17 LogMAR respectively. Using repeated measures ANOVA test, p value for patients receiving intravitreal Ranibizumab+modified grid laser compared for BCVA at 1st follow up, 2nd follow up and 3rd follow up over 6 months , with the baseline was found to be statistically significant (p=0.005).Hence, there was significant improvement in BCVA in patients treated with intravitreal Ranibizumab at 1st follow up, 2nd follow up and 3rd follow up over 6 months, when compared with baseline BCVA.

The RESOLVE study[32], which was a 12 month multicenter randomized study, with the aim to explore the safety and efficacy of intravitreal in diabetic type 1 and type 2 patients with > 300um of DME involving the fovea. It concluded that significant and continuous improvements were noted with the use of Ranibizumab over 12 months as compared with sham treatment. The study used laser photocoagulation as rescue therapy (starting at 3rd month). The limitation of this study however was that it did not assess the visual outcome after the rescue therapy.

The RESTORE study[35] enrolled 345 patients of DME to compare the effects secondary to laser treatment, Ranibizumab injection or combination of the two. Patients received three initial consecutive monthly injections, and laser was performed at baseline. Retreatments were given in accordance with ETDRS guidelines. After 12 months, significant improvement was noted in BCVA in patients with Ranibizumab or combination therapy as compared to laser therapy alone. Also the central retinal thickness changed significantly in the same group of patients.

BOTH GROUPS COMPARISON DISCUSSION:

On comparison of both groups, it was found that mean CMT at the start of the treatment i.e. the baseline, at 1st follow up and at 2nd follow up was statistically insignificant in between and within both the groups (p=0.78, p=0.55 and p=0.08 respectively) but it became significant at the 3rd follow up over a 6 month period (p=0.0001, using one way ANOVA). Therefore there is a significant difference in the improvement achieved at 3rd follow up in CMT within and in between both the groups, over 6 months. This could be due to the fact that initially, treatment with 1st and 2nd monthly doses of Injection Ranibizumab remained the same initially at 1st and 2nd follow ups in both the groups, which made the difference in CMT between both the two groups statistically insignificant initially , but when modified grid laser or additional Inj. Ranibizumab was added to the treatment in the respective groups following the 3rd dose of Injection Ranibizumab, the difference in the CMT between the two groups became statistically significant over 6 months(3rd follow up).

So, at the end of the study we concluded that multiple injections of 0.05 ml (0.5 mg) Ranibizumab (upto 3 doses at monthly intervals) followed by modified macular grid laser treatment (at 7-10 days later) had the best effect on improvement of Central Macular Thickness in comparison to only multiple injections of 0.05 ml(0.5 mg) Ranibizumab (upto 3 doses at monthly intervals, with as required additional monthly doses), over 6 months follow-up.

On comparison of both groups, it was found that mean BCVA at the start of the treatment i.e. the baseline, at 1st follow up, 2nd follow up and 3rd follow up was statistically insignificant in between and within both the groups (p=0.2, p=0.9, p=0.7and p=0.8 respectively, using one way ANOVA).Therefore there no significant difference in the improvement achieved over a follow-up period of 6 months in BCVA within and in between both the groups.

Lee SJ et al [31], Das et al [37], Solaiman et al [29] and Lee et al demonstrated that combination of anti-VEGF agents along with laser photocoagulation compared to monotherapy of repeated intravitreous injections of anti-VEGF only, reduce the burden of repeated intravitreal injections of anti-VEGF drugs without affecting visual outcome.

So, at the end of the study we concluded that multiple injections of 0.05 ml (0.5 mg) Ranibizumab (upto 3 doses at monthly intervals) followed by modified macular grid laser treatment (at 7-10 days later) had no difference in the improvement of Best Corrected Visual Acuity in comparison to only multiple injections of 0.05 ml(0.5 mg) Ranibizumab (upto 3 doses at monthly intervals, with as required additional monthly doses), over 6 months follow-up.

The limitations of this study are its small sample size and limited duration of follow-up.

CONCLUSION:

This parallel group comparison trial has shown that a combination of multiple injections (3 consecutive monthly doses) of Intravitreal Ranibizumab followed by modified grid laser therapy 7-10 days later is more effective in reducing central macular thickness in patients with diabetic macular oedema ≥350μm, in comparison to only multiple injections of Ranibizumab(3 consecutive monthly doses+ additional monthly doses ,as required) , but there is no difference in the final best corrected visual acuity attained in between the two groups, when followed over a 6 month period.

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