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Fundus Fluorescein Angiography in Diabetic Retinopathy: Correlation of Angiographic Findings to the Clinical Maculopathy

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Abstract:
Purpose: *To correlate the fundus fluorescein angiographic findings to the severity of diabetic maculopathy in patients with diabetic retinopathy.*
Materials: *A hospital-based, prospective study of 236 eyes with diabetic retinopathy consisted of a detailed anterior segment and fundus examination with fundus fluorescein angiography. Grading of retinopathy and maculopathy was based on the international classification of diabetic retinopathy and macular oedema respectively. Each angiogram was studied for the number, size and location of aneurysms, shape and size of foveal avascular zone and type of leakage. Angiographic findings were correlated to the clinical grades of diabetic maculopathy. P<0.05 was considered statistically significant.*
Results: *Of the 236 eyes studied 166 (70.33 %) eyes had various grades of maculopathy with a statistically high significant association between the mean number of aneurysms located within one disc diameter(DD) zone centred on the fovea and outside, in both the maculopathy and non maculopathy groups (p<0.0001). There was also a statistically significant correlation of the type of leakage with diabetic maculopathy (p<0.0001). No statistically significant correlation could be established between the size of aneurysm and foveal avascular zone with diabetic maculopathy. Severity of maculopathy was correlated to the mean number of aneurysms located within one DD centred on the fovea and outside one disc diameter, foveal avascular zone and leakage and was found to be statistically significant (p<0.0001).*
Conclusion: *In this study we were able to establish a correlation between maculopathy and mean number of aneurysms as well as the type of leakage. With guidelines based on angiography findings early subclinical maculopathy can be tackled.*
Key words: *diabetic maculopathy, fundus fluorescein angiography, diabetic macular edema,*

I. Introduction

Diabetic retinopathy is a leading cause of blindness and visual impairment in both developed and developing countries and is included by WHO in their "VISION 2020" goal.[1] Diabetic maculopathy is the most important cause for visual impairment in patients with diabetic retinopathy. Diabetic macular oedema (DME) develops in 10% of all diabetic population and centre involving macular edema occurs in 4% of the diabetic population.. Up to 30% of these patients develop moderate visual loss.[2]

Fundus fluorescein angiography (FFA) is useful in determining the size and regularity of foveal avascular zone (FAZ), number and location of micro aneurysms, detection of intra retinal microvascular abnormalities (IRMA and early neovascularisation, which can be missed on clinical examination . The aim of this study was to correlate the number, size, and location of aneurysms, size and regularity of FAZ and pattern of fluorescein leakage to the severity of diabetic maculopathy.

II. Materials And Methods

This was a hospital-based cross sectional study from November 2013 to May 2015 with a calculated sample size of 236 eyes. Ethical clearance for the study was obtained from the institution's ethics committee. Convenience sampling was adopted for the study. Patient's ≥ 30 years of age with diabetic retinopathy were included in this study after obtaining a written informed consent. Patients who had undergone macular photocoagulation or Panretinal photocoagulation, intravitreal injections, prior pars plana vitrectomy, media opacity responsible for inadequate fundus view and other causes of macular edema like venous occlusion, epiretinal membrane, vitreomacular traction were excluded from the study.

All the patients underwent a complete ophthalmic work-up which included a detailed history, ocular examination that included Snellen visual acuity converted to logmar units and intraocular pressure assessment using Goldmann applanation tonometry. Pupils were dilated with Itrop Plus eye drops (Cipla Ltd. India - Tropicamide-0.8% and Phenylephrine Hcl-5%) one drop each eye, repeated after 15 minutes. Anterior segment examination was followed by fundus examination with 78 diopter lens (Volk) and binocular indirect

ophthalmoscope (Appasamy) with 20 diopter (Volk) condensing lens. Diabetic retinopathy and maculopathy was clinically classified based on the International classification and each eye was graded (Table1 & 2). Photo documentation of all cases were done using Topcon Retinal camera –GRC 50DX Germany.

Following this, the patients were subjected to FFA. Patient was advised to fast 3 hours prior to the procedure and were made to sit comfortably in front of the fundus camera. An intradermal test dose of the 20% Sodium Fluorescein was given half an hour prior to the procedure. With the anaesthetist in attendance, a 20 gauge (BD VENFLON) cannula was inserted into the anterior cubital vein. Following this 3 ml of 20% sodium fluorescein (Aurolab) was injected intravenously and photographs were taken at various phases of the angiogram.

1. **Aneurysms: size, location and number** (Fig1) and was studied during the arterio-venous phase. The size of the aneurysm was assessed by comparing to the calibre of the artery at the superotemporal disc margin (considered as 60µm) and was documented as <30µm and >30µm. The numbers of aneurysms within an area of 1DD from the centre of the fovea and 1DD outside this area were counted manually.
2. **FAZ:** The size of the FAZ (as compared to the diameter of the optic disc) and regularity of the margin was studied during the arterio venous phase
3. **Leakage**: This was studied during mid-phase and late phase. Presence of focal leakage, diffuse leakage, combined and cystoid leakage was noted. It was considered focal leakage when the leakage was from a single aneurysm, diffuse when the leakage was from dilated capillaries and cystoid when the leakage had a petalloid appearance.

All these features were studied in patients with Diabetic Retinopathy and the data were analyzed for significance using the SPSSS software (Version 17). Chi square test and Fisher's Exact Test were used and $P < 0.0001$ was considered significant.

Table1. International Clinical Diabetic Retinopathy Severity Scale [3]

Proposed disease Severity scale	**Findings observable on dilated Ophthalmoscopy**
No apparent retinopathy	No abnormalities
Mild NPDR	Micro aneurysm present
Moderate NPDR	More than just micro aneurysms but less than severe NPDR
Severe NPDR	Any of the following : more than 20 intraretinal haemorrhages in each four quadrants; definite venous dilatations in 2+ quadrants; prominent intraretinal micro vascular abnormalities in 1+ quadrant; and no signs of PDR
PDR	One or more of the following: neovascularization, vitreous/ Preretinal haemorrhage

Table 2. International Clinical Diabetic Macular edema Severity Scale [3]

Proposed disease Severity scale	**Findings observable on dilated Ophthalmoscopy**
DME absent	No apparent retinal thickening or hard exudates in posterior pole
Mild DME	Some retinal thickening or hard exudates in posterior pole but distant from the center of the macula
Moderate DME	Retinal thickening or hard exudates approaching the centre of the macula but not involving the centre
Severe DME	Retinal thickening or hard exudates involving the centre of the macula

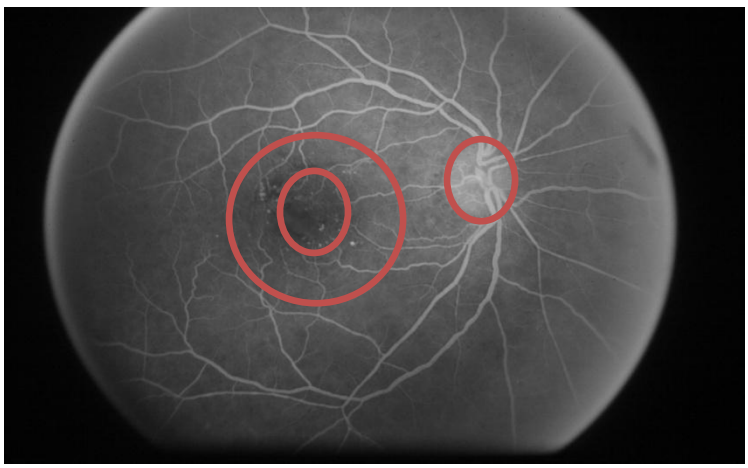

Fig.1 Method adopted for counting aneurysms

III. Results

A total of 121 patients were recruited in this study of which six eyes were excluded as they did not meet the inclusion criteria. Hence 236 eyes was the total sample size of which there were 119 right eyes and 117 left eyes. Out of the 236 eyes, 166 eyes had maculopathy.

There were 29 females and 92 males. The youngest participant was 30 years and the oldest was 75 years. The mean age was 55.51 ± 8.39 years. The mean age of males was 55.53± 8.67 years and the mean age of females was 55.75 ± 7.59 years. The youngest and oldest male participant was aged 30 and 75yrs respectively. Likewise, the youngest and oldest female participant was aged 40 and 65 years respectively. In each age group there were more males as compared to females and both the groups were age matched. (Table 3& Figure2)

Table 3: Age gender distribution of the sample

Age		**Gender**		**Total**
		Female	**Male**	
30 – 39 years	Count	0	2	2
	%	0%	100%	100.0%
40 – 49 years	Count	7	23	30
	%	23.33%	76.66%	100.0%
50 – 59 years	Count	9	38	47
	%	19.14%	80.85%	100.0%
60 – 69 years	Count	13	24	37
	%	35.13%	64.86%	100.0%
70 – 79 years	Count	0	5	5
	%	0%	100%	100.0%
Total	Count	29	92	121
	%	48.29%	51.71%	100.0%

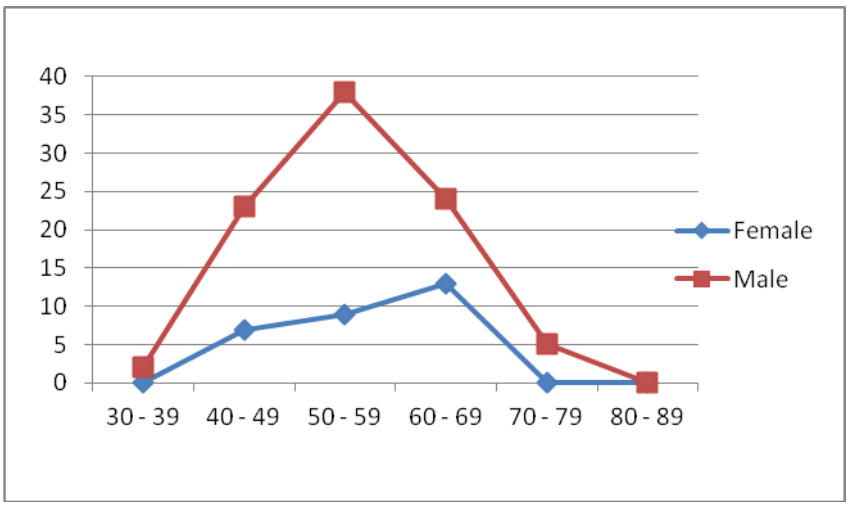

Fig 2: Age gender distribution

114 eyes (48.38%) had moderate Non Proliferative Diabetic Retinopathy(NPDR), followed by 52 eyes (22.08%) with severe NPDR, 35 eyes (14.83%) with Proliferative Diabetic Retinopathy and 35 eyes (14.83%) with mild NPDR. Among these eyes 166 (70.33 %) eyes had maculopathy. (Table4 & Figure 3) The eyes with diabetic maculopathy were further classified into mild, moderate and severe maculopathy and there were 54 eyes with mild maculopathy, 55 eyes with moderate maculopathy and 57 eyes with severe maculopathy.

Table 4: Frequency of diabetic retinopathy and maculopathy

Grades of DR	**Number of eyes**	**Percentage (%)**	**Number of eyes Dmac**	**Percentage of eyes with DR having Dmac**
Mild NPDR	35	14.83%	4	11.42%
Moderate NPDR	114	48.38%	79	69.29%
Severe NPDR	52	22.08%	50	96.15%
PDR	35	14.83 %	33	94.28%
Total	236	100%	166	70.33%

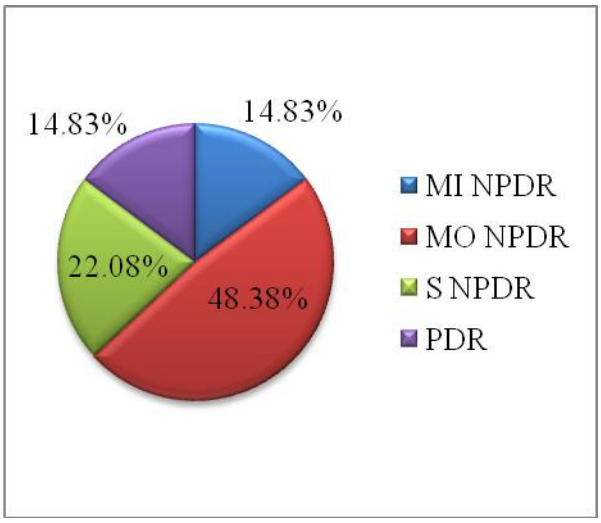

Fig 3: Frequency of grades of diabetic retinopathy among total eyes

The size, number and location of the aneurysms were studied on FFA. The aneurysms were divided into <30µm and >30µm size and the number of aneurysms present within an area of 1 DD centred on the fovea and outside this area were counted. In the non maculopathy group the mean number of aneurysms within 1DD centred on the fovea and outside 1DD was 4.7±3.32and 8.57±6.78 respectively. In the maculopathy group the mean number of aneurysms located within 1DD centred on the fovea and outside 1DD was 9.88±7.36and 17.08±11.86 respectively. (Table 5) The 95% confidence interval for mean number of aneurysms within 1DD centred on the fovea was 3.90 to 5.49 in the non maculopathy group and 6.95 to 10.18 in the maculopathy group.

Table 5: Mean number of aneurysms

Location	Maculopathy Status	Total number	Mean	Std. Deviation
≤1DD	Absent	70	4.70	3.32
	Present	166	9.88	7.36
>1DD	Absent	70	8.57	6.78
	Present	166	17.08	11.86

The difference in the mean number of aneurysms located outside 1DD area centred on the fovea as compared to the mean number of aneurysms within 1DD area was statistically significant in both the maculopathy group and the non-maculopathy groups ($p<0.0001$). For purpose of analysis, the median of number of aneurysms was considered as 12 for aneurysms outside 1DD and 6 for within 1DDcentred on the fovea. There was a significantly high association between the aneurysms located within 1DD centred on the fovea and diabetic maculopathy ($p=0.00002$) and aneurysms located outside 1DD and diabetic maculopathy ($p<0.0001$).

The size of the aneurysm was studied in the two groups. The total number of eyes with aneurysms ≤ 30µm size was 63 and >30µm size was 7 in the non maculopathy group while there were 140 eyes with aneurysms ≤ 30µm size and 26 eyes with aneurysms >30µmsize in the maculopathy group. (Table 6& Figure4) The size of the aneurysm was correlated with maculopathy and no statistical significance was found between them using Fisher exact test ($p=0.174$). Hence it was concluded that maculopathy is independent of the size of the aneurysm.

Table 6: Correlation of size of aneurysms to maculopathy

		Aneurysms		Total
		≤30 µm	>30 µm	
Maculopathy	Absent	63	7	70
	Present	140	26	166
Total		203	33	236

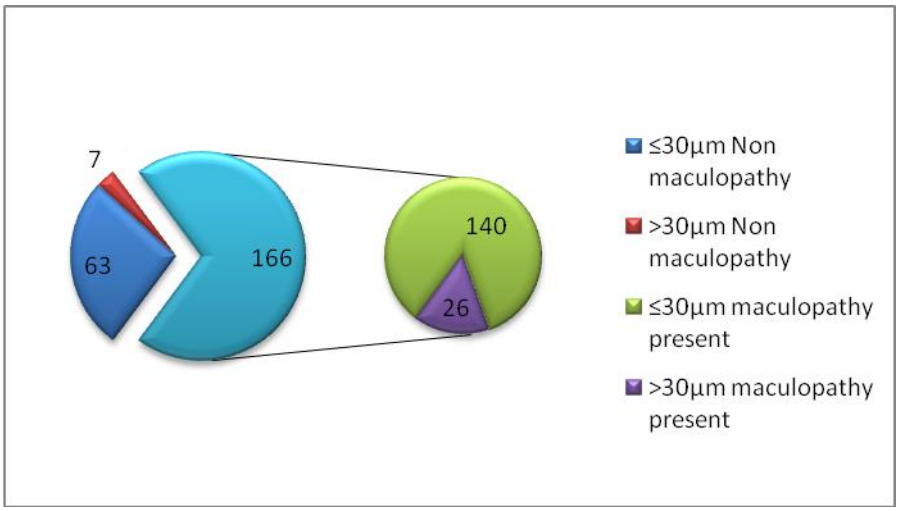

Fig 4: Correlation of the size of aneurysms to maculopathy

The location of the aneurysms was correlated to the severity of maculopathy. Considering the median 6, the total number of eyes with ≤6 aneurysms located within 1 DD were 124 and >6 were 112. Among the mild maculopathy 31 eyes (25%) had ≤6 aneurysms while 23 eyes (20.5%) had >6 aneurysms. In the moderate maculopathy group 18 eyes (14.5%) had ≤6 aneurysms and 37 eyes (33%) had >6aneurysms, and in the severe maculopathy group 20 eyes (16.1%) had ≤6 aneurysms and 37 eyes (33.33%) had a > 6 aneurysms. There was a significant association between the severity of maculopathy and the number of aneurysms located within 1 DD centred on the fovea using chi square test (P<0.0001)

Considering the median 12, the total number of eyes with ≤12 aneurysms located outside 1 DD were 140 and >12 were 96. Among the mild maculopathy 35 eyes (25%) had ≤12 aneurysms while 19 eyes (19.8%) had >12aneurysms. In the moderate maculopathy group 23 eyes (16.4%) had ≤12 aneurysms and 32 eyes (33.3%) had >12aneurysms, and among the severe maculopathy 25 eyes (17.9%) had ≤12 aneurysms and 32 eyes (33.33%) had above 12 aneurysms. There was a significant association between the severity of maculopathy and the number of aneurysms located outside1 DD centred on the fovea using chi square test (P<0.0001) (Table 7 & 8)

Table 7: Correlation of frequency of aneurysms located within 1DD to grades of maculopathy

			Aneurysms		Total
			≤6	>6	
Grades	Absent	Count	55	15	70
		% within Aneurysms	44.4%	13.4%	29.7%
	Mild	Count	31	23	54
		% within Aneurysms	25.0%	20.5%	22.9%
	Moderate	Count	18	37	55
		% within Aneurysms	14.5%	33.0%	23.3%
	Severe	Count	20	37	57
		% within Aneurysms	16.1%	33.0%	24.2%
Total		Count	124	112	236
		% within Aneurysms	100.0%	100.0%	100.0%

Table 8: Correlation of frequency of aneurysms located outside 1DD to grades of maculopathy

			Aneurysms		Total
			≤12	>12	
Grades	Absent	Count	57	13	70
		% within Aneurysms	40.7%	13.5%	29.7%
	Mild	Count	35	19	54
		% within Aneurysms	25.0%	19.8%	22.9%
	Moderate	Count	23	32	55
		% within Aneurysms	16.4%	33.3%	23.3%
	Severe	Count	25	32	57
		% within Aneurysms	17.9%	33.3%	24.2%
Total		Count	140	96	236
		% within Aneurysms	100.0%	100.0%	100.0%

The FAZ was studied for margins and size. In the non maculopathy group 62 eyes had regular margins of the FAZ <1/3DD in size while 8 eyes had distorted margins >1/3DD in size. (Table9& Figure 5) In the maculopathy group 129 eyes had regular margins of the FAZ <1/3DDand 45 eyes had distorted margins>1/3DD in size. There was no statistical significance obtained for correlation of margin and size of FAZ with diabetic maculopathy using Fisher exact test (p=0.036). Hence it was concluded that maculopathy is independent of the margin of FAZ. (Table 9 & Figure 5)

Table 9. Correlation of the margin of FAZ to maculopathy

		Margins		Total
		Regular	Distorted	
Maculopathy	Absent	62	8	70
	Present	129	37	166
Total		191	45	236

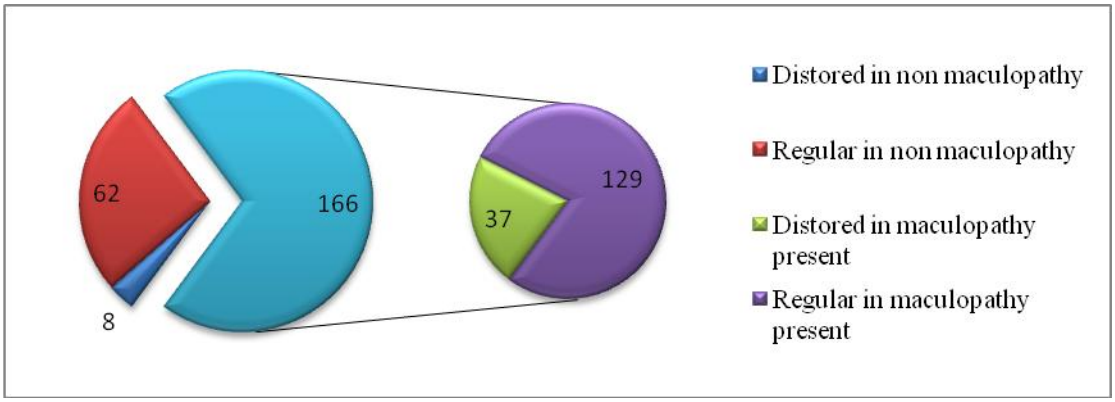

Fig. 5: Correlation of margin of FAZ to maculopathy

FAZ was correlated to severity of maculopathy. In the mild maculopathy group 48 eyes (25.4%) had regular FAZ while 6 eyes (12.8%) had distorted borders. In the moderate maculopathy 42 eyes (22.2%) had regular FAZ and 13 eyes (27.7%) had distorted borders and among the severe maculopathy 30 eyes (15.9%) had regular FAZ and 27 eyes (57.4%) had distorted borders. (Table 10) A highly significant association was obtained between FAZ and severity of diabetic maculopathy. (P<0.0001)

Table 10: Correlation of FAZ to grades of maculopathy

			FAZ		Total
			Regular	Distorted	
Grades	Absent	Count	69	1	70
		% within FAZ	36.5%	2.1%	29.7%
	Mild	Count	48	6	54
		% within FAZ	25.4%	12.8%	22.9%
	Moderate	Count	42	13	55
		% within FAZ	22.2%	27.7%	23.3%
	Severe	Count	30	27	57
		% within FAZ	15.9%	57.4%	24.2%
Total		Count	189	47	236
		% within FAZ	100.0%	100.0%	100.0%

Leakage was studied under three headings focal, diffuse and cystoid leakage. Among the 236 eyes 96 eyes had diffuse leakage, 138 eyes had focal leakage and two eyes had cystoid leakage. Of the 166 eye with maculopathy two eyes had cystoid leakage while 82 eyes each had focal and diffuse leakage respectively. Of the eyes among the non maculopathy group, none had cystoid leakage, while 14 eyes had diffuse and 56 eyes had focal leakage. For the purpose of analysis the eyes with cystoid leakage were added to diffuse leakage. (Table 11) The type of leakage was correlated with maculopathy using Fisher exact test and there was a significant correlation of type of leakage with diabetic maculopathy (P<0.0001)

Table 11. Frequency of leakage among both the groups

		Leakage		Total
		Diffuse	Focal	
Maculopathy	Absent	14	56	70
	Present	84	82	166
Total		98	138	236

Leakage was correlated to the severity of maculopathy. Among the 54 eyes with mild maculopathy 42 eyes (30.4%) had focal leakage and 12 eyes (12.2%) had diffuse leakage. Among the 55 eyes with moderate maculopathy 19 eyes (13.8%) had focal leakage and 36 eyes (36.6%) had diffuse leakage and among the 57 eyes with severe maculopathy 20 eyes (14.5%) had focal leakage and 37 eyes (37.8%) had diffuse leakage. There was a significantly high association between severity of maculopathy and type of leakage (p<0.0001) (Table 12)

Table 12: Correlation of frequency of leakage to grades of maculopathy

			Leakage Type		Total
			Focal	Diffuse	
Grades	Absent	Count	57	13	70
		% within Leakage Type	41.3%	13.3%	29.7%
	Mild	Count	42	12	54
		% within Leakage Type	30.4%	12.2%	22.9%
	Moderate	Count	19	36	55
		% within Leakage Type	13.8%	36.7%	23.3%
	Severe	Count	20	37	57
		% within Leakage Type	14.5%	37.8%	24.2%
Total		Count	138	98	236
		% within Leakage Type	100.0%	100.0%	100.0%

IV. Discussion

Diabetes mellitus is a condition in which the blood glucose levels are elevated. It mainly affects the small vessels and involvement of the retina is quiet common. It can affect the overall quality of an individual's life with marked morbidity. Diabetic retinopathy according to Early Treatment of Diabetic Retinopathy Study (ETDRS) has been classified as mild, moderate, severe and very severe NPDR and proliferative DR.[4] Diabetic maculopathy is one of the components of DR which has a direct effect on vision. This can be missed clinically hence early detection and treatment is necessary. Earlier the modalities of management of diabetic maculopathy were few, but today it has become a great challenge for the ophthalmologist to manage DME with the availability of newer modalities of investigation and treatment. This study was carried out to understand the role of micro aneurysms, their size, location and function in the pathogenesis and severity of diabetic maculopathy.

The aim of this study was to correlate the fundus fluorescein angiographic findings to the presence and severity of clinical maculopathy. We found a high significant association of $p<0.0001$ between the mean number of aneurysms located outside 1DD centered on the fovea and within1DD in both maculopathy and non maculopathy groups while there was no correlation found between the size of aneurysm and diabetic maculopathy. There was no statistical significance obtained for shape and size of FAZ and diabetic maculopathy while there was a significant correlation with type of leakage and diabetic maculopathy.

A Pubmed search with terms like number of aneurysms, FAZ and leakage revealed no results. A study on these findings of FFA in diabetic retinopathy has not been done earlier. Our study is unique as we have tried to find a correlation between the various features of aneurysms as visualised on FFA with clinical maculopathy.

Mehboob *et al* (2015) had done a study on FFA findings among 200 eyes with DME. He found 140(70%) eyes had diffuse leakage, 36 eyes (18%) had focal and 24 (12%) eyes had ischemic maculopathy.[5] A similar interventional study done by Syed SH *et al*(2009) on 130 patients with diabetic retinopathy also established diffuse maculopathy in 77 (59.24%) eyes, focal maculopathy in 23 (17.69%) eyes and ischemic maculopathy in 15(11.55%) eyes while one eye had no maculopathy.[6] In our study among the 236 eyes with DR, 138 eyes (58.47%) had focal leakage, 96 eyes (40.67%) had diffuse leakage, and 2 eyes (0.84%) had cystoid leakage**.** The frequency of eyes with focal maculopathy was more than diffuse maculopathy in our study unlike previous studies. Rajappa A.S *et al*(2014) in his hospital based study among 50 patients to assess and evaluate the role of fluorescein angiography as a tool in the diagnosis of macular disorders found 17 cases of diabetic maculopathy of which 17.6% cases were focal, 25.52% were diffuse and 29.4% were ischemic maculopathy. He also found that FFA confirmed the clinical diagnosis of maculopathy in 23.53% cases, while in 76.47% cases FFA altered the diagnosis and played an important role in categorizing the type of diabetic maculopathy and helped in further management. Hence he concluded that in diabetic maculopathy FFA was a valuable tool in diagnosis of ischemic maculopathy where there is an increase in the foveal avascular zone.[7] Wysles *et al*(1990) however found that FFA helped in confirming the diagnosis in only 40% cases of diabetic maculopathy.[8]

The only limitation of the study is, we have not correlated the FFA findings with Optical Coherence tomography which is one of the most important investigative modality to quantify macular edema

V. Conclusion

It is a fact that better and accurate the diagnosis, the more exact will be the treatment plan. So diagnostic measures should aim towards accuracy for an effective treatment regimen.

In this study we were able to establish a correlation between maculopathy and mean number of aneurysms as well as the type of leakage. Thus this could be useful in the early detection and treatment of subclinical maculopathy. Guidelines can be framed based on the FFA findings for the early treatment of subclinical maculopathy.

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