A Study on the Role of Hyperhomocysteinemia in Diabetic Macular Edema in Type 2 Diabetes Mellitus

Dr. Liza Kuli[1], Dr. Tapan Gogoi[2], Dr. Monigopa Das[3], Dr. Abhijit Kr. Handique[4] [1]Post Graduate Trainee, Dept. of Ophthalmology, Assam Medical College, Dibrugarh

[2]Professor & HOD, Dept. of Ophthalmology, Assam Medical College, Dibrugarh

[3]Professor, Dept. of Biochemistry, Assam Medical College, Dibrugarh

[4]AssistantProfessor, Dept. of Ophthalmology, Assam Medical College, Dibrugarh

ABSTRACT –
AIM: To determine the relationship between serum homocysteine concentration and diabetic macular edema in patients with type 2 diabetes, attending Department of Ophthalmology of Assam Medical College and Hospital, Dibrugarh.
MATERIALS AND METHODS: Patients with type 2 diabetes with diabetic retinopathy having macular edema (n = 30) were enrolled in a cross-sectional hospital-based study. The study period was 6 months. Diabetic macular edema status was documented by fundus photographs and central macular thickness (CMT) was measured by optical coherence tomography (OCT). Serum homocysteine concentration was measured using enzyme immunoassay (ELISA). Hyperhomocysteinemia was defined when homocysteine levels were higher than 15µmol/L.
RESULTS : A significant relationship (p<0.0001) was found between severity of diabetic macular edema based on central macular thickness and serum homocysteine levels, as well as with severity of hyperhomocysteinemia and diabetic macular edema (p<0.0001). Majority of patients had moderate DME with intermediate hyperhomocysteinemia, with mean hcy levels of 35.54±3.00µmol/L, establishing a significant association.
CONCLUSION : Increased levels of homocysteine may explain the role of vascular dysregulation and endothelial dysfunction in patients with diabetic macular edema. Further prospective studies with larger sample size and longer follow ups are necessary to clarify thiscausation.

I. INTRODUCTION

Diabetes mellitus has emerged as one of the most common, consequential chronic diseases of recent times, causing life threatening, disabling complications.(1) Diabetic retinopathy (DR) is a microvascular complication of diabetes, which is caused by damage to blood vessels, resulting in retinal ischemia and increased permeability. New blood vessel formation (neovascularization) and diabetic macular edema (DME) are common characteristics for the disesase.(2)

Diabetic macular edema (DME), which is characterised by increased vascular permeability and hard exudate deposition at the central retina, can occur at any stage of DR. Diabetic macular edema (DME) is theleading cause of poor visual acuity in diabetic patients.(3)

The most commonly used parameter to evaluate DME for management and prognosis is central macular thickness (CMT).(3)It ispossible to measure macular thickness objectively and track the progression of DME quantitatively using optical coherence tomography (OCT).(4) Spectral domain OCT (SD OCT) is an imaging modality that helps in diagnostic evaluation of DME patients and aids in understanding the precise anatomic alterations and pathophysiology of DME.(3)

In recent years, hyperhomocysteinemia has been postulated as a potential risk factor for development and progression of retinopathy in

patients with diabetes. Homocysteine (Hcy) is a sulphur-containing amino acid formed by the demethylation of the dietary amino acid, methionine.

Higherblood levels of homocysteine are considered toxic to the vascular endothelium through generation of free radicals, impairs platelet activity and increases smooth muscle proliferation. Free radicals cause disruption of endothelial integrity, leading to platelet activation,causing hypercoagulability and thrombus formation.(5)

Several studies have been conducted worldwide to investigate the role of hyperhomocysteinemia in DR. Some of these studies have found an association between hyperhomocysteinemiaand diabetes-induced microangiopathies (diabetic nephropathy, diabetic retinopathy and macular edema). (5-12)

However, certain studies have not come to a fruitful conclusion regarding the association between hyperhomocysteinemia and diabetic retinopathy.(13,14)

In our study, we assess the role of hyperhomocysteinemia in diabetic retinopathy, especially in patients with diabetic macular edema.

CLASSIFICATION OF DIABETIC RETINOPATHY

The most commonly used classification of DR is the Modified Airlie House Classification, which was introduced by the Early Treatment Diabetic Retinopathy Study (ETDRS).(15) Diabetic macular edema is an important determinant of visual function in diabetics, and can be present either with non-proliferative or proliferative diabetic retinopathy.

| **Nonproliferative Diabetic Retinopathy (NPDR)**	
A.	Mild NPDR
	At least one microaneurysm
	Definition not met for B, C, D, E, F
B.	Moderate NPDR
	H/Ma ? standard photograph No. 2A
	Soft exudates, VB, and IRMA definitely present
	Definition not met for C, D, E, F
C.	Severe NPDR
	H/Ma ? standard photograph No. 2A (Fig. 133.1) in all 4 quadrants
	VB in 2 or more quadrants (Fig. 133.3)
	IRMA > standard photograph No. 8A in at least 1 quadrant (Fig. 133.2)
D.	Very Severe NPDR
	Any two or more of C.
	Definition not met for E, F
Proliferative Diabetic Retinopathy (PDR)	
	(Composed of:(1) NVD or NVE, (2) preretinal or vitreous hemorrhage, (3) fibrous tissue proliferation)
E.	Early PDR
	New vessels
	Definition not met for F
F.	High-risk PDR
	NVD (1/3 - 1/2 disc area (Fig. 133.4) or
	NVD and vitreous or preretinal or vitreous hemorrhage (Fig. 133.5) or
	NVE ? ½ disc area and vitreous or preretinalhemorrhage
Clinically Significant Macular Edema (CSME)	
1.	Thickening of the retina at or within 500 ?m from the center of the macula or
2.	Hard exudates with thickening of the adjacent retina located at or within 500 mm from the center of the macula or
3.	A zone of retinal thickening, 1 disc area or larger in size located at or within 1 disc diameter from the center of the macula.
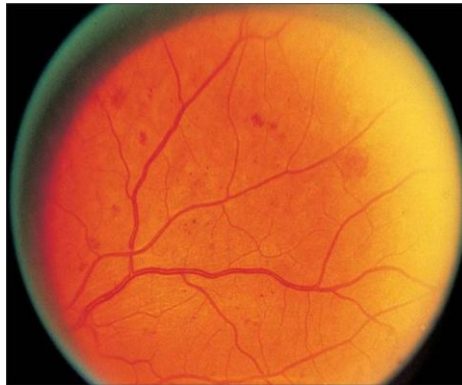
Fig : 1 - Standard photograph No. 2A of the Modified Airlie House Classification of Diabetic Retinopathy demonstrating a moderate degree of hemorrhage or microaneurysms, or both. (Source : Albert &Jakobiec's Principles & Practice of Ophthalmology, 3rd edition, Chapter 133)
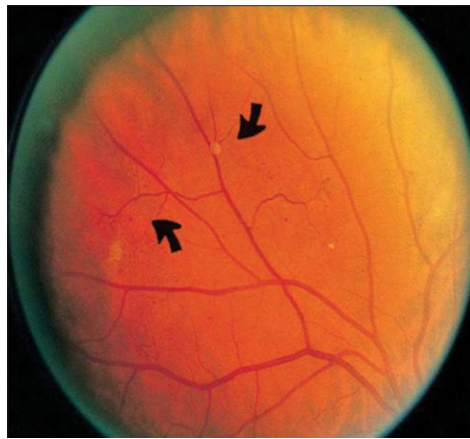

Fig : 2 - Standard photograph No. 8A of the Modified Airlie House Classification of Diabetic Retinopathy demonstrating intraretinal microvascular abnormalities (IRMAs) (arrows). (Source : Albert &Jakobiec's Principles & Practice of Ophthalmology, 3rd edition, Chapter 133)

DIABETIC MACULOPATHY

Diabetic macular edema is defined as retinal thickening within one disc diameters of the macula's centre. It can be focal, diffuse, or ischemic in nature. ETDRS classified DME patients as having clinically significant macular edema (CSME) or not. (15)
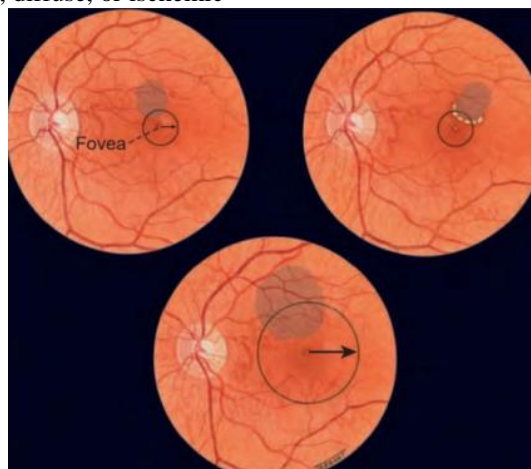

Fig :3 - Clinically significant macular oedema (Source : Brad Bowling. Retinal vascular disease-Diabetic retinopathy. Kanski's Clinical Ophthalmology-A Systematic Aproach. 8 th ed. Sydney, Australia.Elsevier; 2016.p: 527.)

CSME includes any one of the following lesion:
1. Retinal thickening at or within 500 microns from the center of macula.
2. Hard exudates at or within 500 microns from center of macula associated with thickening of the adjacent retina.
3. An area or areas of retinal thickening at least one disc area in size, at least a part of which is within one disc diameter of the center of macula.

Based on Central Macular Thickness (CMT), the patients of Diabetic Macular Edema were further subdivided into 3 groups. The Classification was done based on Time-domain OCT (TD-OCT) value. The values obtained were of SD-OCT which were converted to TD-OCT using the formula,
TD-OCT value = −43.12 + 1.01 × SD-OCT value. (16)

(1) Mild (CMT: 201–300 µm)
(2) Moderate (CMT : 301 – 400 µm)
(3) Severe (CMT> 400 µm)

CLASSIFICATION OF HYPERHOMOCYSTEINEMIA (17)
Hyperhomocysteinemia has been classified as –
(1) Moderate (15–30 µmol/L)
(2) Intermediate (31–100 µmol/L)
(3) Severe (>100 µmol/L), based on serum Hcy levels

II. MATERIALS AND METHODOLOGY

AIM AND OBJECTIVE :
To assess the relationship between serum homocysteine levels and diabetic macular edema in type 2 diabetics.

METHODOLOGY :
We selected 30 type 2 diabetes mellitus patients with diabetic macular edema, attending Ophthalmology OPD of Assam Medical College and Hospital.
Type of study – Hospital-based cross-sectional study
Place of study – Assam Medical College & Hospital, Dibrugarh
Study duration – 6 months
Study population– All patients above 40 years attending Ophthalmology OPD diagnosed to have type 2 diabetes mellitus with diabetic macularedema

Inclusion Criteria:
1. Patients of either sex, age > 40 years
2. All diabetic patients having diabetic Retinopathy with diabetic macular edema
3. Type 2 diabetes mellitus

Exclusion Criteria:
1. Patients with type 1 diabetes mellitus

2. Patients with pre-existing non-diabetic maculopathy (like that due to central serous retinopathy, age related macular degeneration, drug induced and macular degeneration)
3. Hazy media (not allowing examination of fundus)
4. Previous history of retinal laser photocoagulation
5. Pregnancy, females on oral contraceptives or hormone therapy
6. Patients with degenerative or dystrophic conditions of the retina
7. Patients taking vitamin supplementations or medications known to affect serum homocysteine concentrations, such as theophylline,statins, fibrates, levodopa, PPIs, anticonvulsants etc
8. Patients with a history of vascular disease (myocardial infarct or angina, stroke etc), kidney disease,familial hypercholesterolemia, hypothyroidism, chronic liver disease, neoplasms, dementia etc

INSTRUMENTS USED:
1. Snellen'sVisual Acuity Chart
2. Slit lamp biomicroscopy using +90D lens
3. Direct Ophthalmoscope (Heine Beta 200 LED)
4. Indirect ophthalmoscope (Heine Omega 250) with +20D lens
5. Zeiss VISUCAM-500 fundus camera
6. Fundus fluorescein angiography, whenever indicated
7. SD OCT (Zeiss Cirrus)

ETHICAL CONSIDERATION :
The study proposal was submitted in the Institutional Ethics Committee of Assam Medical College and Hospital, Dibrugarh for review and appraisal and the study was commenced after approval.

CONSENT :
A written and informed consent was taken from the participants for conducting the study.

DIAGNOSIS:
The participants underwent thorough clinical evaluation including history, general physical examination, systemic examination & local ophthalmological examination.
Best corrected visual acuity (Snellen's Visual Acuity Chart) was recorded. Dilated fundus examination was done 5-15 minutes after instillation of mydriatic-cycloplegic eye drop (5% Phenylephrine hydrochloride & 0.8% Tropicamide) with direct ophthalmoscope followed by bimanual

Indirect Ophthalmoscope using Volk +20D aspheric lens and Slit Lamp examination with Volk +90D lens.

- Direct Ophthalmoscopy: It was done in a semi dark room. Once the retina was focused, fundus details were examined systematically starting from the optic disc, blood vessels, periphery of the retina, all four quadrants of the general background for any pathology and finally concluded with the macula.
- Indirect ophthalmoscopy with +20D lens: The fundus details in each eye were observed with a stereoscopic view. All the quadrants of the fundus were examined and findings drawn on a fundus chart. Scleral indentation was done to visualise the whole of peripheral retina up to oraserrata.

- Slit Lamp examination with +90D lens: Examination done under highly magnified, 3-dimensional view of optic disc/posterior segment. Cases identified with macular edema were then evaluated by doing OCT macula. Digital Fundus Photograph (DFP) and Fundus fluorescein angiography (FFA) were done whenever indicated.

Diabetic macular edema were graded based on Central macular thickness (CMT) values obtained from OCT. Blood investigations like fasting blood sugar (FBS), postprandial blood sugar (PPBS),glycosylatedhemoglobin (HbA1c) levels and serum homocysteine levels were done. We then tried to find the relationship between serum homocysteine levels and diabetic macular edema.

III. RESULTS

Table 1: DISTRIBUTION OF DME CASES IN DIFFERENT AGE GROUPS

AGE GROUP (in years)	NUMBER (n)	PERCENTAGE (%)
41-50	2	6.6
51-60	16	53.3
61-70	10	33.3
>70	2	6.6
TOTAL	30	100

Out of 30 patients with DME, majority (53.3%) were between 51-60 years of age, while 6.6% were between the age groups 41-50 years, 33.3% belonged to 61-70 years and 6.6% were >70 years.
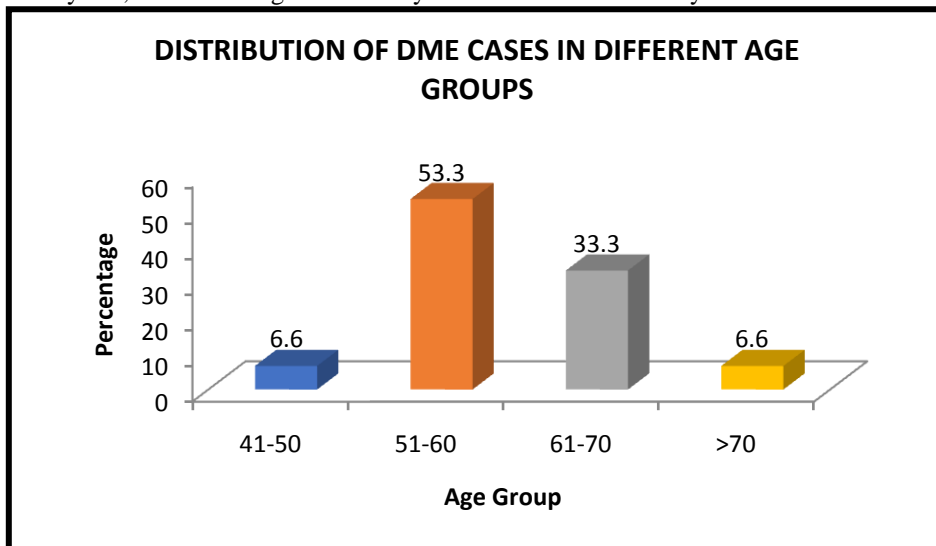

Table 2 : DISTRIBUTION OF DME CASES IN DIFFERENT GENDER OF THE PATIENTS

GENDER	NUMBER (n)	PERCENTAGE (%)	RATIO (Male : Female)
Male	19	63.3	1.7 : 1
Female	11	36.6	
Total	30	100	

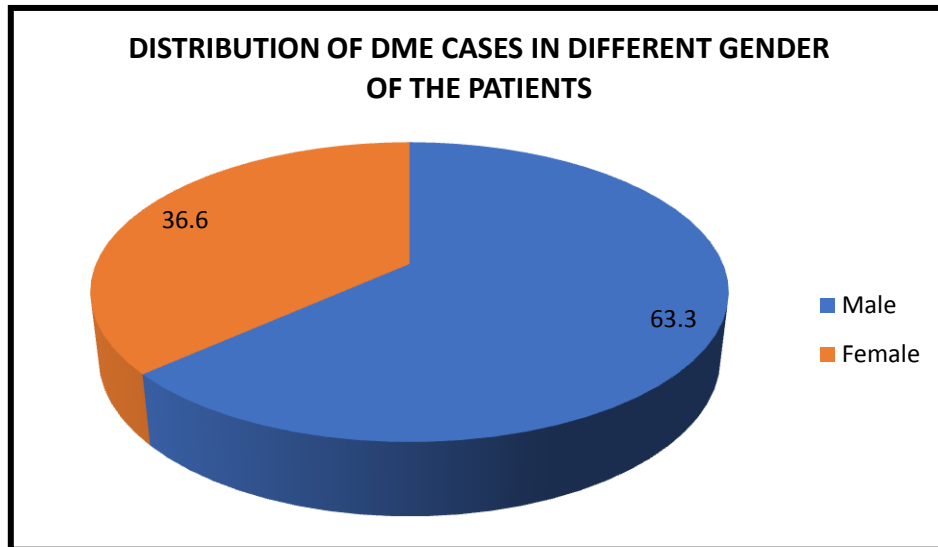Out of 30 patients, majority (63.3%) of them were males and 36.6% were females, with male : female ratio of 1.7 : 1.

DISTRIBUTION OF DME CASES IN DIFFERENT GENDER OF THE PATIENTS

TABLE 3 : DISTRIBUTION OF DME CASE ACCORDING TO DURATION OF DIABETES

DURATION (in years)	NUMBER (n)	PERCENTAGE (%)
5-10	10	33.3
11-20	16	53.3
>20	4	13.3
Mean±S.D.	14.37±5.08	

In our study, majority (53.3%) of DME patients had a duration of diabetes between 11-20 years, followed by 33.3% patients with duration of DM 5-10 years and 13.3% having diabetes for >20 years.
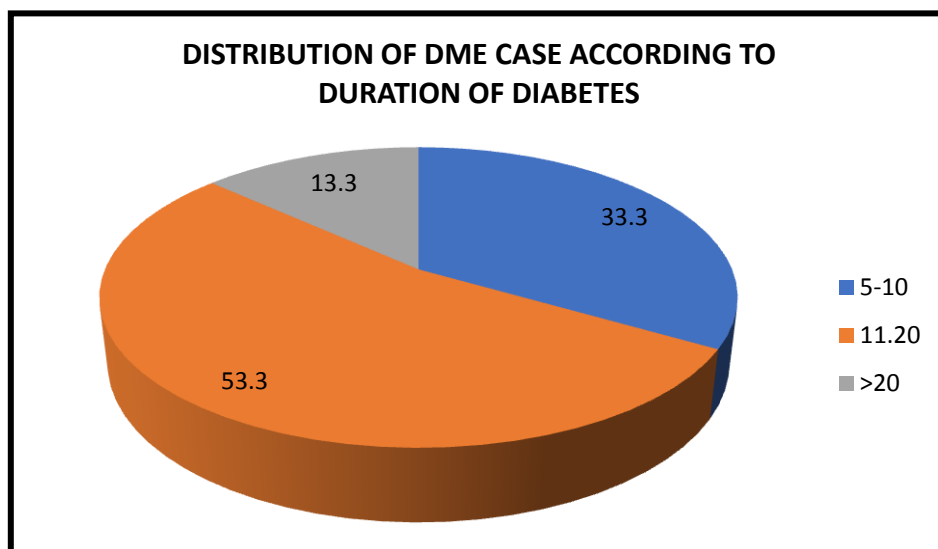The mean duration of diabetes was 14.37±5.08 years.

DISTRIBUTION OF DME CASE ACCORDING TO DURATION OF DIABETES

TABLE 4 : : DISTRIBUTION OF DME CASES ACCORDING TO TYPE OF TREATMENT

TREATMENT	NUMBER (n)	PERCENTAGE (%)
OHA	23	76.6
Insulin	7	23.3
TOTAL	30	100

Out of 30 patients, majority (76.6%) of the patients were on oral hypoglycaemic agents and only 23.3% were on insulin.
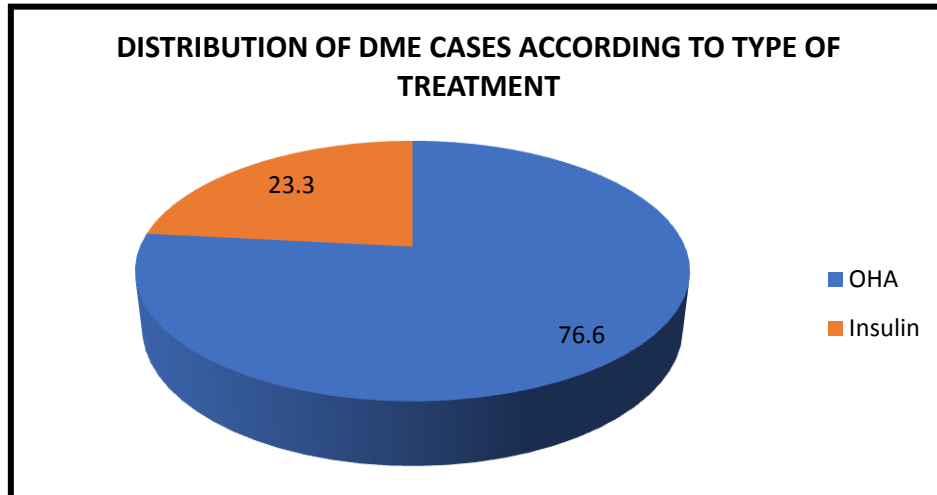

TABLE 5 : RELATIONSHIP OF GRADES OF DIABETIC RETINOPATHY WITH DME AND CMT (Central Macular Thickness)

GRADES OF RETINOPATHY	DME		CMT (Mean ± S.D.) (µm)	p-value
	NUMBER (n = 30)	PERCENTAGE (%)		
Mild NPDR	0	0	0	0.0003
Moderate NPDR	8	26.6	479.42±143.63	
Severe NPDR	10	33.3	274.25±21.74	
PDR	12	40	374.40±58.81	

Out of the 30 patients with diabetic macular edema, maximum number of patients were seen in PDR (40%) followed by 33.3% of patients inSevere NPDR and in Moderate NPDR there was 26.6% of patients and no patient in Mild NPDR.Mean CMT of patients with moderate NPDR was 479.42±143.63 µm, that of severe NPDR was 274.25±21.74µm and of PDR was 374.40±58.81 µm.

There was a significant relationship ($p<0.05$) between the grades of diabetic retinopathy and the central macular thickness of the patients with diabetic macular edema.
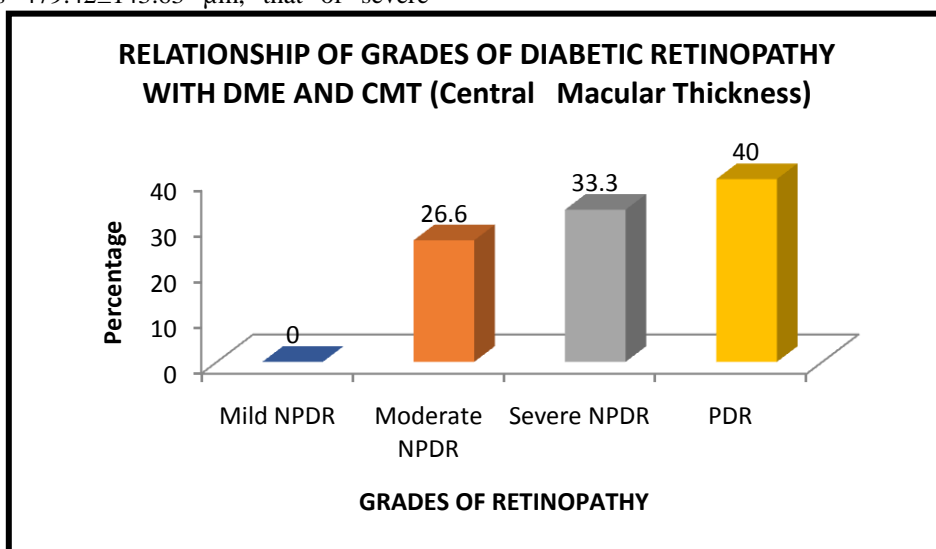
TABLE 6 : RELATIONSHIP BETWEEN SEVERITY OF DME BASED ON CMT WITH HOMOCYSTEINE LEVEL

SEVERITY OF DME BASED ON CMT	NUMBER (n=30)	PERCENTAGE (%)	SERUM HOMOCYSTEINE (Mean ± S.D.) (µmol/L)	p-value
Mild (201–300 µm)	10	33.3	15.51±3.03	<0.0001
Moderate (301–400 µm)	12	40	29.13±6.13	
Severe (> 400 µm)	8	26.6	36.39±2.97	

Majority (40%) of patients had moderate DME with mean serum homocysteine of 29.13±6.13µmol/L, 33.3% patients had mild DME and mean serum homocysteine of 15.51±3.03µmol/L, while 26.6% had severe DME with mean serum homocysteine of 36.39±2.97µmol/L.

In our study, we found a significant relationship ($p<0.05$) between severity of diabetic macular edema based on central macular thickness and serum homocysteine levels.
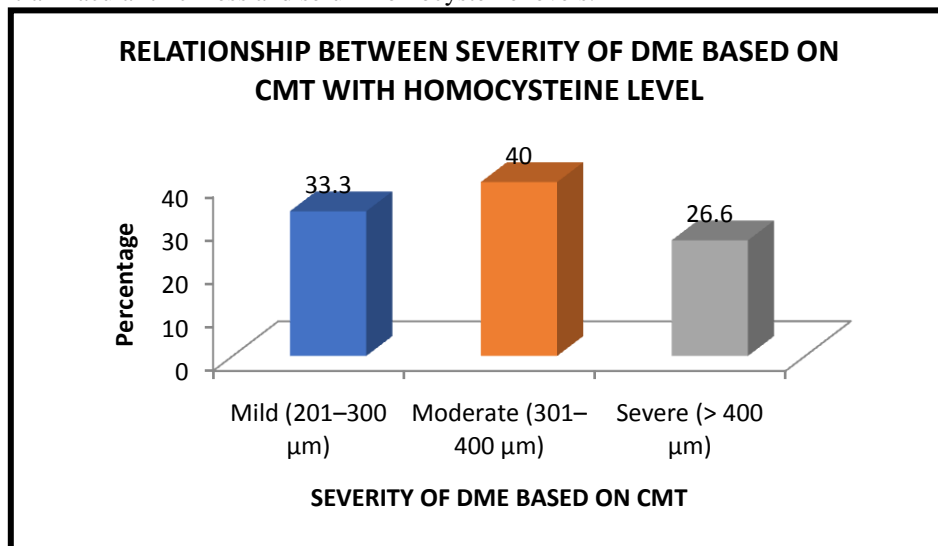

TABLE 7 : RELATIONSHIP BETWEEN DME WITH HOMOCYSTEINE LEVEL

SERUM HOMOCYSTEINE (µmol/L)	NUMBER (n)	PERCENTAGE (%)	Mean±S.D
Normal (<15)	5	16.6	12.86±0.85
Moderate Hyperhomocysteinemia (15-30)	11	36.6	21.27±3.70
Intermediate Hyperhomocysteinemia(30-100)	14	46.6	35.54±3.00
Severe Hyperhomocysteinemia (>100)	----	----	----
TOTAL	30	100	26.53±9.52
p-value	<0.0001		

Majority of the diabetic macular edema patients (46.6%) had intermediate hyperhomocysteinemia with mean serum hcy of 35.54±3.00µmol/L, followed by 36.6% having moderate hyperhomocysteinemiawith mean hcy21.27±3.70µmol/Land 16.6% with normal homocysteine levels, with a mean hcy of 12.86±0.85 µmol/L. The overall mean homocysteine level in the DMEpatients found was 26.53±9.52µmol/L.

The relationship between DME and serum homocysteine was found to be significant with $p<0.05$.

RELATIONSHIP BETWEEN DME WITH HOMOCYSTEINE LEVEL
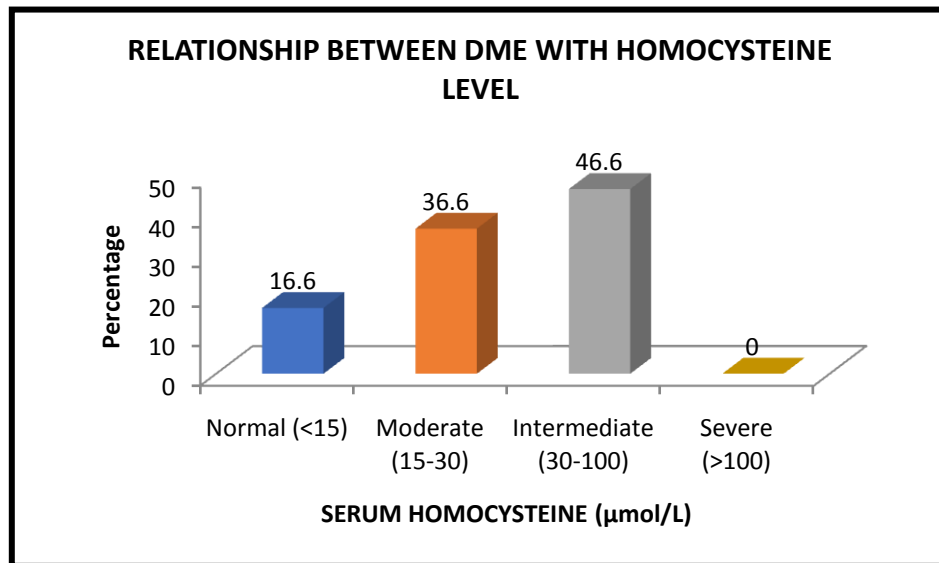
IV. DISCUSSION

In our present study, out of 30 patients with DME, majority (53.3%) were between 51-60 years of age, while 6.6% were between the age groups 41-50 years, 33.3% belonged to 61-70 years and 6.6% were >70 years. The study population of Brazionis et al. (median age of 66.5 years in DR) and Fotiou et al. (median of 68 years in DR) were much older compared to our study population.(7,18)

Majority (63.3%) of the participants were males and 36.6% were females. Similar findings was found in a study by M. Goldstein et al. (5), with 51.39 % males and 48.60% females.

In our study, the mean duration of diabetes was 14.37±5.08 years.Fotiou et al found raised hcy levels in patients with duration of diabetes ≥ 16 years.(18)

76.6% of the patients were on oral hypoglycaemic agents and only 23.3% were on insulin. The results were similar to a study by Sato et al, (19) who found marginally higher homocysteine levels in metformin users as compared with metformin non-users.

In our study, maximum number of patients were seen in PDR (40%) followed by 33.3% of patients in Severe NPDR and in Moderate NPDR there was 26.6% of patients and no patient in Mild NPDR.

Majority (40%) of patients had moderate DME with mean serum homocysteine of 29.13±6.13 µmol/L, 33.3% patients had mild DME and mean serum homocysteine of 15.51±3.03µmol/L, while 26.6% had severe DME with mean serum homocysteine of 36.39±2.97µmol/L. In a study by N Dong et al, higher homocysteine levels were associated with an increased central subfield macular thickness, average macular thickness and average macular volume in diabetic patients without DME, which may indicate that patients with type 2 diabetes with increased levels of plasma tHcy are more prone to develop a clinical manifestation of DME.(20)

Majority of the diabetic macular edema patients (46.6%) had intermediate hyperhomocysteinemia with mean serum hcy of 35.54±3.00µmol/L, followed by 36.6% having moderate hyperhomocysteinemiawith mean hcy21.27±3.70µmol/L and 16.6% with normal homocysteine levels, with a mean hcy of 12.86±0.85 µmol/L. The overall mean homocysteine level in the DMEpatients found was 26.53±9.52 µmol/L. A study by Aydin et al revealed mild to moderate elevation of homocysteine that may explain the role of vascular dysregulation and endothelial dysfunction in patients with DR.(9)

V. CONCLUSION

Our study showed a significant relationship between diabetic macular edema and serum homocysteine levels, majority having moderate DME and intermediate hyperhomocysteinemia. There was also a steady increase in serum homocysteine levels with increasing severity of diabetic retinopathy in patients with type 2 diabetes. Patients with PDR had higher serum homocysteine levels.

Homocysteinecould be a potential diagnostic marker for diabetic macular edema in patients with diabetic retinopathy, to predict the incidence and severity of retinal damage in diabetic patients. Targeting the clearance of homocysteine

could also be a therapeutic target for diabetic retinopathy with macular edema.

From this study, it can be inferred that all patients with type 2 diabetes mellitus, besides undergoing fundoscopic examination, should be assessed for serum homocysteine status and supplemented appropriately, so as to enhance Hcy clearance and prevent or even retard the progression of diabetic macular edema.

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