International Journal of Biomedical Research
ISSN: 0976-9633 (Online); 2455-0566 (Print)
Journal DOI: https://doi.org/10.7439/ijbr
CODEN: IJBRFA

Original Research Article

A study of plasma homocysteine levels in type 2 diabetes mellitus and its correlation with diabetic retinopathy

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Abstract

Introduction: Diabetic Retinopathy is an ocular manifestation of diabetes, a systemic disease, which affects up to 80 percent of all patients who have had diabetes for 20 years or more. Homocysteine (Hcy) is a highly reactive Thiol-containing amino acid derived from the conversion of methionine to cysteine, producing reactive oxygen species – hydrogen peroxide and upper oxide anion radical. Findings may have implications for treatment of hyperhomocysteinemia to prevent further vascular damage & micro complications in type 2 Diabetes Mellitus.

Aims and objectives: To find correlation between plasma homocysteine (tHcy) values and Type 2 Diabetes with and without Retinopathy.

Materials and methods: It s a single centre Case control study. Three groups will be selected. In study group, 50 patients of newly detected diabetes mellitus or known case of diabetes mellitus will undergo fundoscopy and those with NPDR and PDR and normal RFT will be categorised in group 1. 50 patients of newly detected diabetes mellitus or known case of diabetes mellitus with normalfundoscopy, RFT are included in group 2 (selected by random sampling procedure). 50 Control group involving non diabetic individuals with normal renal parameters and No other medical conditions mentioned in exclusion criteria will be selected randomly and categorised in to group plasma homocysteine levels of all the three groups are tabulated and compared.

Results: In DM group, mean Homocysteine was 14.90±5.59, in DR group, mean Homocysteine was 38.86±7.69 and in controls, mean Homocysteine was 10.61±1.46. There was significant difference in mean plasma Homocysteine between three groups. (p value <0.001).

Conclusion: Mean (±SD) level of plasma homocysteine was higher among the DR group (NPDR) as compared to the DM group and controls, the difference was statistically significant.

Keywords: Case control study, Diabetic Retinopathy, Homocysteine, Reactive oxygen species, vascular damage.

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***Article History:**
Received: 03/07/2019
Revised: 04/08/2019
Accepted: 18/08/2019
DOI: https://doi.org/10.7439/ijbr.v10i8.5221

QR Code

How to cite: Bande U. S. and Chethan Y. A study of plasma homocysteine levels in type 2 diabetes mellitus and its correlation with diabetic retinopathy. *International Journal of Biomedical Research* 2019; 10(08): e5221. DOI: 10.7439/ijbr.v10i8.5221 Available from: https://ssjournals.com/index.php/ijbr/article/view/5221

1. Introduction

Diabetes mellitus is a group of metabolic diseases characterized by persistent hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycaemia of diabetes is associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. The vast majority of diabetes falls in two broad categories. In one category, Type 1 (autoimmune) diabetes, the cause is an absolute deficiency of insulin secretion. The next category, the more prevalent one, Type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response [1].

The worldwide prevalence of diabetes has risen dramatically over the past two decades, from an estimated 30 million cases in 1985 to 285 million in 2010. Based on current trends, the International Diabetes Federation projects that 438 million individuals will have diabetes by the year 2030. [1]

India has the second largest diabetic population in the world. The recently published ICMR national study reported that there are 62.4 million people with type 2 diabetes (T2DM) and 77 million people with pre-diabetes in India. These numbers are projected to increase to 101 million by the year 2030, unless urgent preventive steps are

taken, [1] hence there is urgent need to prevent diabetes and its complications.

The various Micro vascular complications in diabetes mellitus include [1]:

- Diabetic retinopathy
- Diabetic nephropathy
- Diabetic neuropathy

Micro vascular complications of diabetes are associated with considerable medical and economic impact among persons with diabetes. According to, the prevalence of microvascular complications in diabetes in South Asia (India) is-39.0%, renal disease is 20.3%, eye disease is 16.3% and neuropathy is 24.6%. Prevalence of microvascular complications of diabetes in south Indian population, according to the CUPS (Chennai Urban Population Study) and CURES (Chennai Urban Rural Epidemiological Study) by Mohan *et al*[2] is diabetic retinopathy-17.6%, microalbiminuria -26.9%, overt nephropathy-2.2% and diabetic neuropathy-17.5%.

1.1 Need for study

Diabetic Retinopathy is an ocular manifestation of diabetes, asystemic disease, which affects up to 80 percent of all patients who have had diabetes for 20 years or more.[3] Despite these intimidating statistics, research indicates that at least 90% of these new cases could be reduced if there were proper and vigilant treatment and monitoring of the eyes.[1] The longer a person has diabetes, the higher his or her chances of developing diabetic retinopathy.

1.2 Risk factors for diabetic retinopathy include:

- People with type 1 or type 2 diabetes are at risk for developing diabetic retinopathy. The longer a person has diabetes, the more likely he or she is to develop diabetic retinopathy, particularly if the diabetes is poorly controlled.
- Hispanics and African Americans are at greater risk for developing diabetic retinopathy [4].
- People with other medical conditions, such as high blood pressure and high cholesterol, are at greater risk [4]
- Pregnant women face a higher risk for developing diabetes and diabetic retinopathy. If a woman develops gestational diabetes, she has a higher risk of developing diabetes as she ages.[4]
- There are several factors other than hyperglycemia causing vascular complications. One of these risk factors is homocysteine (Hcy). [1]
- Homocysteine (Hcy) is a highly reactive thiol-containing amino acid derived from the conversion of methionine to cysteine, producing reactive oxygen species – hydrogen peroxide and upper oxide anion radical [1].
- Elevated plasma Hcy is vasculotoxic as it causes endothelial dysfunction, increased oxidative Stress, altered coagulation, fibrinolysis, smooth muscle cell proliferation, and changes in Structural and elastic properties of vessel wall
- It also limits nitric oxide (NO) production and promotes lipid per oxidation thereby decreasing the bio availability of NO [2].
- In diabetes the pro-oxidative state worsens by auto-oxidation of Hcy leading additional oxidative stress and thereby to endothelial dysfunction, platelet activation and thrombus.
- Many studies have established the relationship between homocysteine levels and diabetic Macrocvascular complications. Not many Indian studies have dealt with the association between homocysteinemia & Diabetic Retinopathy.
- Studies have evaluated the Diabetic retinopathy and homocysteine levels but have yielded Inconsistent results.[5-7]
- Findings may have implications for treatment of hyperhomocysteinemia to prevent further vascular damage & micro and macrovascular complications in type 2 diabetes mellitus. Hence this study was undertaken to determine the role of homocysteine levels in diabetic retinopathy.

2. Material and methods

2.1 Study Settings: This study was conducted in Tertiary care Hospital, KIMS, Hubli

2.2 Study Population: Patients with type 2 diabetes mellitus and diabetic retinopathy admitted in KIMS Hospital, Hubli.

2.3 Inclusion criteria

- Patients with type 2 diabetes mellitus with diabetic retinopathy
- Age >40 years
- Both male and female sex

2.4 Exclusion criteria:

- Pregnancy
- Renal failure
- Stroke
- MI in last three months
- Diagnosed malignancies
- Alcohol ingestion
- Patients taking drugs like methotrexare, fibric acid derivative and niacin, phenytoin, carbamazepine,
- Megaloblastic anemia

2.5 Study Design: case control study [Diabetics with Retinopathy, Diabetics without Retinopathy and Non Diabetics as controls]

2.6 Study Duration: 01/12/2016 to 31/12/2018.

2.7 Sampling Technique: Purposive sampling technique was used to include the subjects.

2.8 Sample Size

Was estimated by using the difference in Mean difference in Homocystenine levels among diabetic and controls group from the doctorate thesis by Giulia

Malaguarnera *et al* as 12.1±6.8 and 7.8±6.4. Using these values at 95% Confidence limit and 80% power sample size of 38 was obtained in each group by using the below mentioned formula and Med calc sample size software. With 20% non response sample size of 38 + 7.6 ≈ 46 rounded of to 50 subjects in each group were included. Hence a total of 150 were required in the study. However 54 diabetic retinopathy cases were obtained in the study period and were included in the study.

2.9 Sample Size Estimation Formula:

$$\text{Sample size} = \frac{2SD^2(Z_{\alpha/2}+Z_{\beta})^2}{d^2}$$

SD – Standard deviation = From previous studies or pilot study

$Z_{\alpha/2} = Z_{0.05/2} = Z_{0.025} = 1.96$ (From Z table) at type 1 error of 5%

$Z_{\beta} = Z_{0.20} = 0.842$ (From Z table) at 80% power

d = effect size = difference between mean values

So now formula will be

$$\text{Sample size} = \frac{2SD^2(1.96 + 0.84)^2}{d^2}$$

Zα at 95% confidence level (1% alpha error) = 1.96
Zβ at 80% power = 0.84

2.10 Methods of collection of data

In the study 3 groups were selected. Among them, 2 were study groups and 1 control group.

2.11 Study Group:

Group 1: 50 patients of known case of diabetes mellitus and newly detected diabetes mellitus will undergo fundoscopy and those with NPDR and PDR and normal RFT were categorised in to Group 1.

Group 2: 50 patients of known case of diabetes mellitus and newly detected diabetes mellitus with Normal fundoscopy and normal RFT were included in group 2.

Control Group: 50 Control group involving non diabetic individuals with normal renal parameters and no other medical conditions mentioned in exclusion criteria were selected and categorised in to group 3.

Pre structured questionnaire was used to collect the data from patients. The questionnaire had the details related to socio demographic profile of the patients, clinical examination findings and laboratory findings. All standards techniques were used to collect the data. FBS, PPBS, RFT Electrolytes, USG Abdomen, Urine routine and Serum Homocysteine were the laboratory investigations done among subjects.

All patients were provided the information sheet regarding the study. Information sheets were available in English, Hindi and Tamil. For illiterate participants, the information sheet was read out in the language understood by them. Patients were then enrolled in the study after obtaining their informed consent.

A detailed questionnaire was administered to all the participants of the study. Measurement of blood pressure (BP) and estimation of Body mass index was done for all participants of the study. Ophthalmological investigations like Fundus fluorescein angiography and Optical coherence tomography, which were required for the routine management of diabetic retinopathy, were done as per standard clinical indications.

Fasting (AC) and two-hour postprandial blood sugar levels (PC), glycosylated hemoglobin (HbA1c), lipid profile (LDL levels), hemoglobin (Hb) and serum creatinine, if done within the past three months, were recorded. If these investigations were not available in the past three months, they were done as part of the standard of care for the management of diabetic patients.

A fasting venous blood sample was collected from all participants for the estimation of serum homocysteine. Green tube containing heparinized lithium was used for sample collection, and serum homocysteine was analyzed by competitive immunoassay, using direct chemiluminescent technology.

2.12 Method used for Homocysteine estimation

ADIVA Centaur HYC competitive immunoassay. This method uses direct chemiluminescent technology for homocysteine estimation in the serum.

2.13 Potential confounders/ suspected effect modifiers

Data regarding confounding factors/ suspected effect modifiers were obtained by history/ clinical examination/ laboratory investigations. The following were considered as potential confounders/ suspected effect modifiers:

Age and gender were matched between cases and controls. Other factors like duration of diabetes, glycemic control, hypertension, anemia, hyperlipidemia, nephropathy, smoking, alcoholism, obesity and analyzed.

2.14 Management of bias

The laboratory personnel who processed and analyzed the blood samples were masked towards the group to which the patient belonged (whether case or control).

2.15 Diagnostic criteria/ definitions

Diagnostic criteria for DM (According to American Diagnostic criteria)

- FBS- >126mg/dl
- OGTT with 2hr post load value >200mg/dl
- RBS>200mg/dl with symptoms such as polyuria, polydipsia, polyphagia
- HbA1c>6.5%

Diagnostic criteria for diabetic retinopathy [8]

Non proliferative diabetic retinopathy (NPDR):

Mild- Indicated by the presence of at least 1 micro aneurysm

Moderate- Includes the presence of haemorrhages, micro aneurysms, and hard exudates

Severe- Characterized by haemorrhages and micro aneurysms in 4 quadrants, with venous beading in at least 2 quadrants and intraretinal microvascular abnormalities in at least 1 quadrant

2.16 Proliferative diabetic retinopathy (PDR):

***Neovascularisation*:** Hallmark of PDR [8]

***Preretinal haemorrhages*:** Appear as pockets of blood within the potential space between the retina and the posterior hyaloid face.

***Haemorrhage into the vitreous*:** May appear as a diffuse haze or as clumps of blood clots within the gel

Fibro vascular tissue proliferation

Traction retinal detachments

2.17 Hyperhomocysteinemia

The normal homocysteine concentrations range from 5 - 15 micromol /L. Hyperhomocysteinemia classification:

Moderate ranges from 15 - 30 micromol/L

Intermediate ranges from 30 - 100 micromol/L

Severe is defined as more than 100 micromol/L

However, as hyperhomocysteinemia is still an emerging risk factor for diabetic retinopathy, different studies have taken different cut off values for hyper-homocysteinemia. Prevalence of hyperhomocysteinemia and odds ratios reported in various studies has also shown wide variation. Therefore, for the purpose of this study, the median serum homocysteine value of >15 µmol/L was taken as the cut off for hyperhomocysteinemia.

2.18 Statistical analysis:

Data was entered into Microsoft excel data sheet and was analyzed using SPSS 22 version software. Categorical data was represented in the form of Frequencies and proportions. Chi-square test was used as test of significance for qualitative data. Continuous data was represented as mean and SD. ANOVA (Analysis of Variance) or Kruskal Wallis test was the test of significance to identify the mean difference between more than two groups for quantitative and qualitative data respectively.

Graphical representation of data: MS Excel and MS word was used to obtain various types of graphs such as bar diagram.

p value (Probability that the result is true) of <0.05 was considered as statistically significant after assuming all the rules of statistical tests.

Statistical software: MS Excel, SPSS version 22 (IBM SPSS Statistics, Somers NY, USA) was used to analyze data.

Ethical Aspects:

Ethical clearance was obtained from the Institutional Ethical Committee prior to the start of the study.

3. Results and observation

In the study 50 Diabetics without Diabetic retinopathy, 54 Diabetics with Diabetic Retinopathy and 50 controls without diabetes were finally included for analysis.

Table 1: Profile of subjects in the study

	Group						P value
	DM		DR		Controls		
	Mean	SD	Mean	SD	Mean	SD	
Age	52.80	13.09	54.22	12.67	50.20	8.99	0.215
BMI	22.58	2.69	23.47	2.70	23.00	2.74	0.251
W/H Ratio	0.98	0.10	0.98	0.11	0.97	0.09	0.929

Among DM group, mean age was 52.80±13.09 years, mean BMI was 22.58±2.69 and mean W/H Ratio was 0.98±0.1, among DR group mean age was 54.22±12.67years, mean BMI was 23.47±2.7 years and mean W/H Ratio was 0.98±0.11. Among controls, mean age was 50.2±8.99 years, mean BMI was 23.00±2.74 and mean W/H Ratio was 0.97±0.09. There was no significant difference in mean age, BMI and W/H Ratio between three groups.

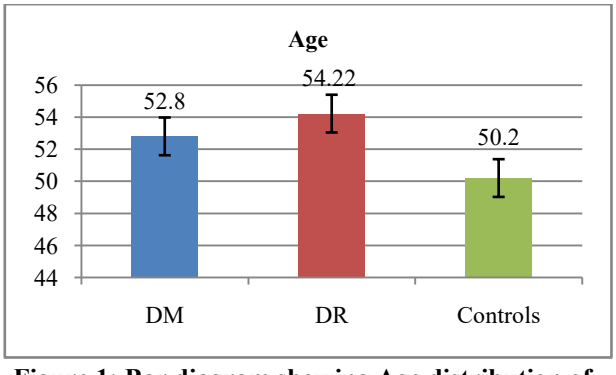

Figure 1: Bar diagram showing Age distribution of subjects

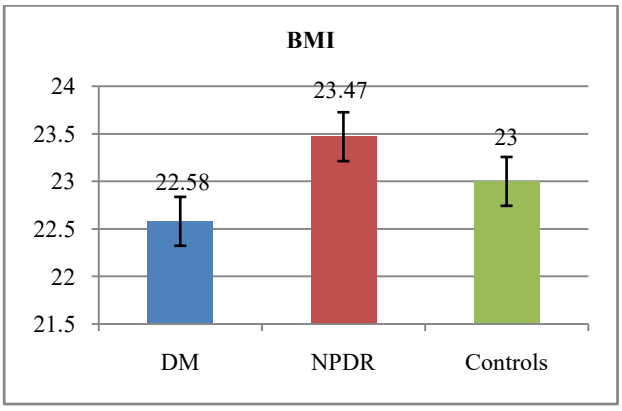

Figure 2: Bar diagram showing BMI distribution of subjects

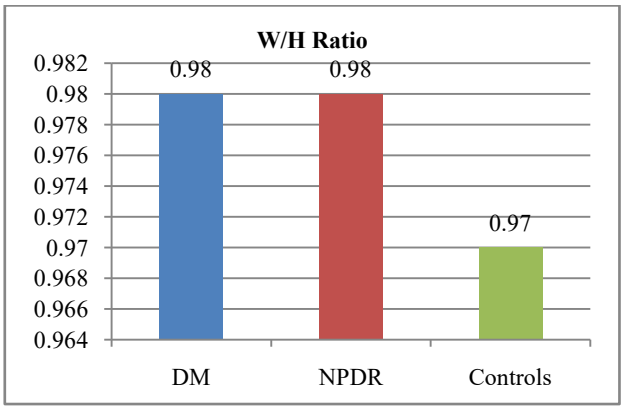

Figure 3: Bar diagram showing Waist Hip Ratio distribution of subjects

Table 2: Gender distribution of subjects

		Group					
		DM		DR		Controls	
		Count	%	Count	%	Count	%
Sex	Male	38	76.0%	36	66.7%	38	76.0%
	Female	12	24.0%	18	33.3%	12	24.0%

$\chi 2 = 1.54$, df = 2, p = 0.463

Among DM group, 76% were males, 24% were females, among DR group, 66.7% were males and 33.3% were females and among Controls, 76% were males and 24% were females. There was no significant difference in gender distribution between three groups.

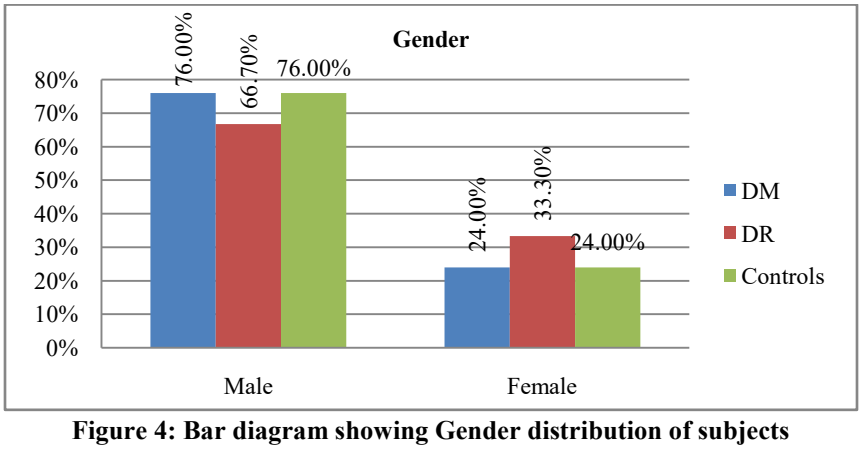

Figure 4: Bar diagram showing Gender distribution of subjects

Table 3: Diabetic profile of subjects among DM subjects and DR group

		Groups				P value
		DM		DR		
		Count	%	Count	%	
Mean duration of DM	Mean±SD	4.80±2.01 years		6.57±2.098 years		<0.001*
Drugs (OHAs/Insulin)	OHA	34	68.0%	36	66.7%	0.218
	Insulin	14	28.0%	11	20.4%	
	Both	2	4.0%	7	13.0%	
Fundus	1	50	100.0%	0	0.0%	<0.001*
	2	0	0.0%	49	90.7%	
	3	0	0.0%	5	9.3%	
Diabetic Foot	Yes	0	0.0%	3	5.6%	0.091
	No	50	100.0%	51	94.4%	
	Group					P value
	DM			DR		
	Mean	SD		Mean	SD	
FBS	139.80	34.27		132.39	19.86	0.176
PPBS	230.78	53.25		214.22	42.05	0.08
HbA1c	7.40	1.84		8.38	2.63	0.032*

Mean duration of DM among DM group was 4.80±2.01 years, 68% were on OHA's, 28% were on Insulin and 4% were on both OHA + Insulin, 0% had Diabetic foot.

Mean duration of DM among DR group was 6.57±2.098 years, 66.7% were on OHA's, 20.4% were on Insulin and 13% were on both OHA + Insulin, 5.6% had Diabetic foot and 90.7% had Grade 1 and 9.3% had Grade 2 DR.

There was significant difference in mean duration of DM and fundal changes between DM and DR group.

In DM group Mean FBS was 139.80±34.27, mean PPBS was 230.78±53.25 mg/dl, Mean HbA1c was 7.40±1.84.

In DR group, Mean FBS was 132.39±19.86, mean PPBS was 214.22±42.05 mg/dl, Mean HbA1c was 8.38±2.63.

There was significant difference in mean HbA1c between two groups.

There was no significant difference in mean FBS and PPBS between two groups

Fundus:

1- Normal fundoscopy,

2- Diabetic Retinopathy

(All patients included in the study were NPDR. As the patients With PDR had diabetic nephropathy, not satisfying the inclusion criteria),

3- Hypertensive Retionopathy.

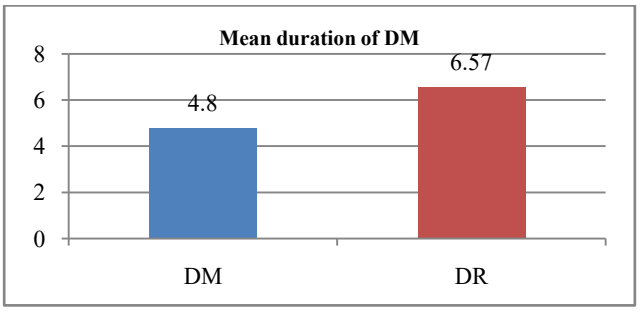

Figure 5: Bar diagram showing Mean duration of DM b/w DM and DR group

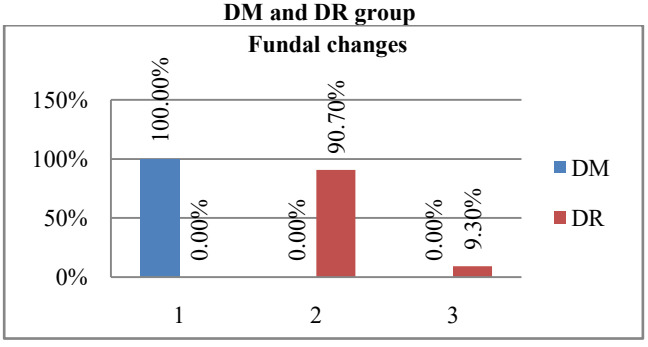

Figure 6: Bar diagram showing Fundus changes between DM b/w DM and DR group

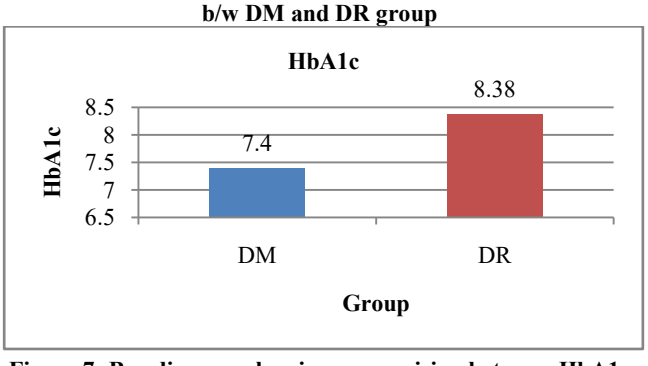

Figure 7: Bar diagram showing comparision between HbA1c of DM group and retinopathy group

Table 4: HTN profile of subjects

		Group					
		DM		DR		Controls	
		Count	%	Count	%	Count	%
HTN	Yes	37	74.0%	30	55.6%	0	0.0%
	No	13	26.0%	24	44.4%	50	100.0%

$\chi 2 = 60.61$, df = 2, p <0.001*

In DM group, 74% had HTN, in DR group, 55.6% had HTN and in controls none of them had HTN. There was significant difference in HTN between three groups.

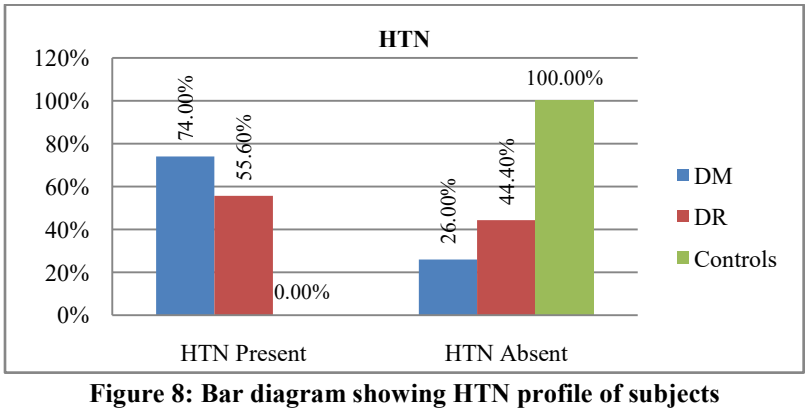

Figure 8: Bar diagram showing HTN profile of subjects

Table 4: Smoking profile of subjects

		Group					
		DM		DR		Controls	
		Count	%	Count	%	Count	%
Smoker	Yes	26	52.0%	19	35.2%	24	48.0%
	No	24	48.0%	35	64.8%	26	52.0%

$\chi 2 = 3.274$, df = 2, p = 0.195

Among DM group, 52% were smokers, among DR group, 35.2% were smokers and among controls 48% were smokers. There was no significant difference in smoking history between cases and controls.

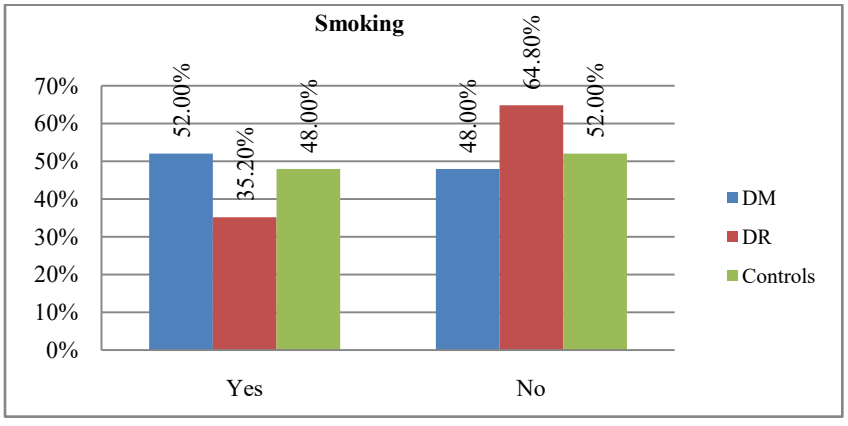

Figure 9: Bar diagram showing Smoking profile of subjects

Individuals included in all the groups were NON alcoholics.

Table 5: ECG findings of subjects

		Group					
		DM		DR		Controls	
		Count	%	Count	%	Count	%
ECG	Normal	50	100.0%	54	100.0%	50	100.0%

All the subjects in three groups had normal ECG.

Table 6: Investigations comparison between cases and controls

	Group						P value
	DM		DR		Controls		
	Mean	SD	Mean	SD	Mean	SD	
FBS	139.80	34.27	132.39	19.86	94.50	10.26	<0.001*
PPBS	230.78	53.25	214.22	42.05	150.82	14.61	<0.001*
HbA1c	7.40	1.84	8.38	2.63	5.39	0.40	<0.001*
Hb%	11.64	1.24	11.08	7.64	12.55	7.66	0.490
TC	8026.36	2623.35	9427.26	9697.52	9076.00	2892.89	0.490
Urea	30.74	6.12	32.72	8.91	31.24	6.03	0.344
Creatinine	1.01	.14	1.09	.42	.99	.16	0.111
Total Cholesterol	183.50	48.11	185.31	41.57	190.34	49.79	0.748
TG	185.22	62.82	181.94	54.60	201.10	66.65	0.245
LDL	91.02	40.36	92.20	38.85	94.46	46.26	0.916
VLDL	45.56	15.40	44.80	13.51	46.92	16.69	0.773
HDL	51.12	13.44	53.41	12.39	52.16	15.71	0.702

Mean FBS in DM group was 139.80±34.27 mg/dl, in DR group was 132.39±19.86 mg/dl and in control group was 94.50±10.26 mg/dl. There was significant difference in mean FBS between three groups.

Mean PPBS in DM group was 230.78±53.25 mg/dl, in DR group was 214.22±42.05 mg/dl and in control group was 150.82±14.61 mg/dl. There was significant difference in mean PPBS between three groups.

Mean HbA1c in DM group was 7.40±1.84, in DR group was 8.38±2.63 and in control group was 5.39±0.40. There was significant difference in mean HbA1c between three groups.

There was no significant difference in mean Hb%, Total count, Total Cholesterol, Urea, Creatinine, TG, LDL, VLDL and HDL between three groups.

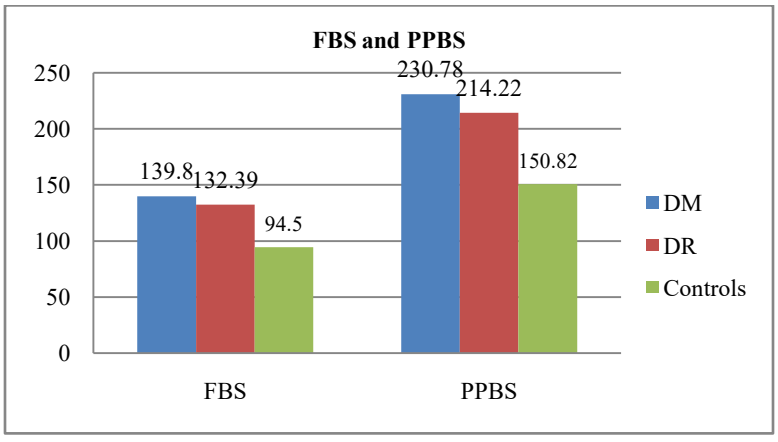

Figure 10: Bar diagram showing FBS and PPBS comparison between cases and controls

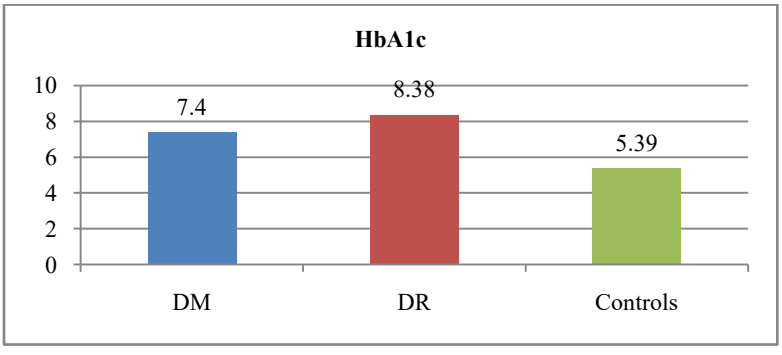

Figure 11: Bar diagram showing HbA1c comparison between cases and controls

Table 7: Urine Routine Findings comparison between three groups

		Group					
		DM		NPDR		Controls	
		Count	%	Count	%	Count	%
Urine routine	Nil	50	100.0%	54	100.0%	50	100.0%

In all the three groups, none of them had abnormal urine routine findings.

Table 8: plasma Homocysteine comparison between three groups

	Mean	SD
DM	14.90	5.59
DR	38.86	7.69
Controls	10.61	1.46
P value	<0.001*	

In DM group, mean Homocysteine was 14.90±5.59, in DR group, mean Homocysteine was 38.86±7.69 and in controls, mean Homocysteine was 10.61±1.46. There was significant difference in mean Serum Homocysteine between three groups.

This suggests, Retinopathy group had higher Serum Homocysteine than DM and control groups.

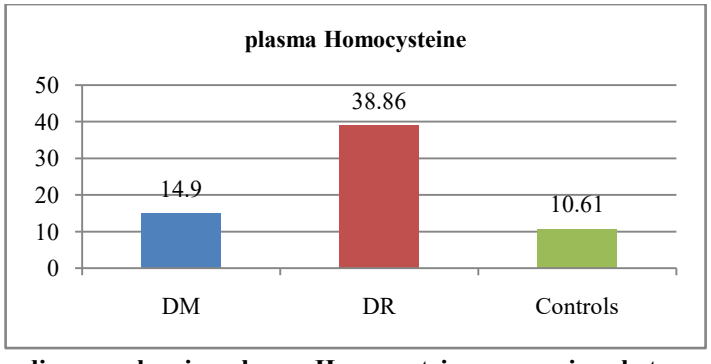

Figure 12: Bar diagram showing plasma Homocysteine comparison between three groups

Table 9: Association between plasma Homocysteine and three groups

		Group					
		DM		DR		Controls	
		Count	%	Count	%	Count	%
Serum Homocysteine	Increased	15	30.0%	54	100.0%	0	0.0%
	Normal	35	70.0%	0	0.0%	50	100.0%

$\chi 2 = 111.542$, df = 2, p <0.001*

In DM group, 30% had increased Serum Homocysteine (>15), in DR group 100% had increased Serum Homocysteine and in Control group 100% had normal Serum Homocysteine. There was significant association between Serum Homocysteine and three groups.

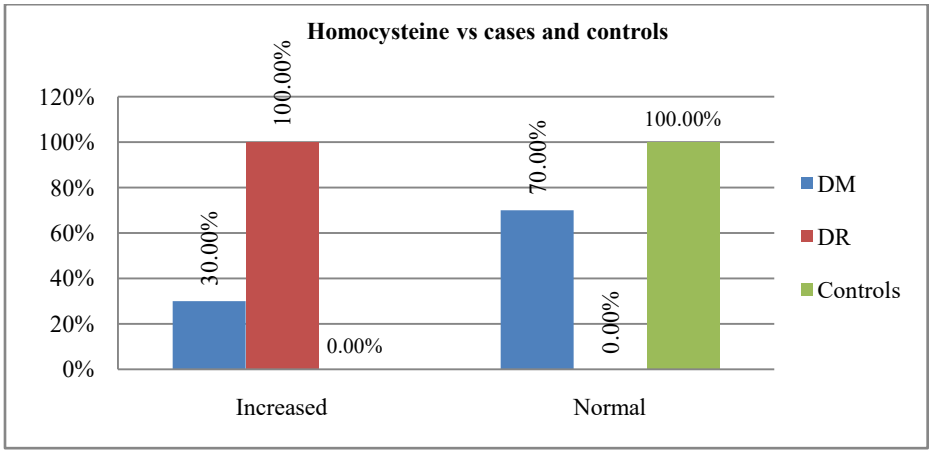

Figure 13: Bar diagram showing Association between Serum Homocysteine and three groups

Table 10: Correlation between Homocysteine and variables between Cases and controls

		Serum Homocysteine	Duration of DM (years)	BMI	W/H Ratio	FBS	PPBS	HbA1c
DM	Pearson Correlation (r)	1	0.226	-0.078	-0.018	0.026	0.038	0.093
	P value		0.114	0.589	0.901	0.859	0.796	0.519
DR	Pearson Correlation (r)	1	0.173	0.130	-0.081	-0.027	0.027	0.127
	P value		0.211	0.350	0.558	0.844	0.848	0.361
Controls	Pearson Correlation (r)	1		-0.176	-0.011	0.079	0.184	-0.072
	P value			0.220	0.941	0.586	0.201	0.621

In all the three groups, there was no significant correlation between Serum Homocysteine and Duration of DM, BMI, W/H Ratio, FBS, PPBS and HbA1c.

4. Discussion

The global prevalence of diabetic retinopathy (DR) among diabetics is 34.6%.[9] With the increasing prevalence of diabetes in India, its ocular complications are also on the rise, The prevalence of diabetic retinopathy, as estimated in the CURES Eye study, was 17.6% among diabetic patients. The prevalence of DR was reported to be 22.4% in southern India as per the Andhra Pradesh Eye Disease Study (APEDS).

DR is one of the leading causes of blindness among the adult population suffering from diabetes. There are several risk factors that are well known for the progression of retinopathy. Some factors like age, duration of diabetes and genetic predisposing factors are non-modifiable. On the other hand, risk factors like hyperglycemia, hypertension, anemia, hyperlipidemia, obesity and nephropathy are modifiable to varying extents. In the last decade, elevated homocysteine level in the blood has emerged as a novel risk factor for the progression and development of DR.[10] However, there are a few studies that have failed to show an association of hyperhomocysteinemia with progression of diabetic retinopathy.[11,12]

Therefore, there has been no definite evidence so far, to prove or disprove this association. Since supplementation of vitamin B12 and folate has been shown to reduce homocysteine levels in serum to varying extents in different studies, hyperhomocysteinemia may be a modifiable risk factor. Hence, understanding and characterizing the role of hyperhomocysteinemia in the pathogenesis of DR may help in identifying a novel target to combat this potentially blindness causing disease.

Although a number of studies have been done to study the association of diabetic retinopathy with hyperhomocysteinemia, the results have not always been consistent. Some studies have found a strong association of

hyperhomocysteinemia with diabetic retinopathy, [13] while others have failed to do so.[14]

These studies have concluded that hyperhomocysteinemia may not be an independent risk factor for diabetic retinopathy, and have suggested that other condition associated with diabetes, like declining renal dysfunction, may cause elevation of serum homocysteine levels in diabetic patients.

4.1 Defining hyperhomocysteinemia

Although hyperhomocysteinemia is being studied as a possible risk factor for ocular microvascular complications, there are disparities between various studies conducted on this subject. The cut-off for defining hyperhomocysteinemia is arbitrary, and has differed substantially among different studies. The global disparity in defining hyperhomocysteinemia may be due to different genetic constitutions, life styles, environmental, nutritional and dietary factors. The cut-off values defined in various studies range from 12 µmol/L - 16 µmol/L. In the present study Hyperhomocysteinemia is defined as serum Homocysteine levels >15micro mol/L.

4.2 Demographic profile

The serum homocysteine levels do depend upon the age profile of patients, as described by Moat *et al*. We, therefore, chose age-matched controls for each of our cases with NPDR. The mean age was 52.80±13.09 years, mean BMI was 22.58±2.69 and mean W/H Ratio was 0.98±0.1, among DR group mean age was 54.22±12.67years respectively, in the study done by Satyanarayana *et al* [14] which was similar to that of our study population (55.3±5.4 years and 54.8±6.1 years in PDR and no DR group respectively).[14] The study populations of Brazionis *et al*. (median age of 66.5 years in DR and 65 years in No DR) and Fotiou *et al*. (median of 68years in DR and 61 years in No DR group) were much older compared to our study population.[14]

Parameters	Our study			Satyanarayana *et al*	
	DM	DR	control	DM	DR
Age	52.80±13.09	54.22±12.67	50.20±8.99	55.34±5.4	54.8±6.1
BMI	22.58±2.69	23.47±2.70	23.00±2.74	-	-
W/H RATIO	0.98±0.1	0.98±0.11	0.97±0.09	-	-

4.3 Gender

Levels of homocysteine are usually higher in men compared to women. Hence, we selected gender-matched controls for each of our cases, to decrease the confounding effect of gender. In our study, Among DM group, 76% were males, 24% were females, among DR group, 66.7% were males and 33.3% were females and among Controls, 76% were males and 24% were females. The studies at Brazionis *et al* and Fotiou *et al* have not looked at gender as a risk factor.[14]

4.4 Risk factors

4.4.1 Duration of diabetes

Longer duration of diabetes has been associated with several macro and micro vascular complications, including retinopathy. Mean duration of DM among DM group was 4.80±2.01 years, 68% were on OHA's, 28% were on Insulin and 4% were on both OHA + Insulin, 0% had Diabetic foot. Mean duration of diabetes among retinopathy group (NPDR) was 6.57±2.098 years, 66.7% were on OHA's, 20.4% were on Insulin and 13% were on both OHA + Insulin. Similarly, the duration of diabetes in the retinopathy group was significantly higher when compared to the no retinopathy group, in the studies done by Brazionis *et al* and Fotiou *et al*.[14] However, the mean duration of diabetes was 11.03±6.9 years vs. 10.16±6.9 years in DR and no DR groups in the study done by Satyanarayana *et al* and the difference was not statistically significant (p = 0.09).[15]

In our study there was no significant correlation between Homocysteine with duration of diabetes in DM Group, DR group.

Fotiou *et al* found significantly increasing median (IQR) serum homocysteine levels with increasing duration of diabetes (11.2 µmol/L (8.7 – 13.9 µmol/L) in patients with duration of diabetes ≤ 5 years vs. 16.9 µmol/L (14.8 – 18.9 µmol/L) in patients with duration of diabetes ≥ 16 years; p < 0.001, and 12.2 µmol/L (10.0 – 16.2 µmol/L) in patients with duration of diabetes 6 – 15 years vs. 16.9 µmol/L (14.8 – 18.9 µmol/L) in patients with duration of diabetes ≥ 16 years; p = 0.001)[19].This study also found a statistically significant difference in serum homocysteine levels between the NPDR and PDR groups (median (IQR) in NPDR group: 15.5 µmol/L (11.8 – 17.4 µmol/L) vs. 18.7 µmol/L (16.5 – 22.0 µmol/L) in the PDR group.[14]

Parameters	Our study			Satyanarayan *et al*		
	DM	DR	P value	DM	DR	P value
Duration of DM	4.80±2.01	6.57±2.09	<0.001	10.16±6.9	11.03±6.9	0.09
HbA1c	7.40±1.84	8.38±2.63	0.032	9±2.5	10.3±2.9	0.01
FBS	139.80±34.27	132.39±19.86	0.176	-	-	-
PPBS	230.78±53.25	214.22±14.05	0.05	-	-	-

4.4.2 Glycemic control

In our study there was significant difference in mean FBS, PPBS and HbA1c between three groups. Higher glycemic levels were observed in DM and DR group compared to Control group.

The mean/ median (with SD/ IQR) HbA1c levels were significantly higher in patients with DR compared to patients without DR in studies done by Satyanarayana *et al*. (10.3±2.9% vs. 9±2.5%; p < 0.01), [14] Brazionis *et al*. [16] (8.6% (7.1 – 10.2%) vs. 7.6% (6.6 – 8.7%); p = 0.003)(11) and Fotiou *et al*.(7.4% – 8.9%) vs. 6.7% (6.0 – 7.6%); p < 0.001).[14]

We also did not find any statistically significant correlation of serum HbA1C levels and homocysteine levels in the three groups

4.4.3 Hypertension

In DM group, 74% had HTN, in DR group, 55.6% had HTN and in controls none of them had HTN. There was significant difference in HTN between three groups. There was no significant difference in the systolic or diastolic blood pressure between the two groups (DR and no DR) in the study done by Brazionis *et al*.[16] However, we found that the prevalence of hypertension was significantly higher in cases as compared to the controls in our study (84.6% vs. 51.3%; p < 0.01).

4.4.4 Smoker

Among DM group, 52% were smokers, among DR group, 35.2% were smokers and among controls 48% were smokers. There was no significant difference in smoking history between cases and controls.

4.4.5 Anemia

In our study there was no significant difference in mean Hb between three groups. Also we did not find a statistically significant correlation of hemoglobin levels with homocysteine levels in any of the three groups.

Satyanarayana *et al* could not demonstrate a significant difference in the mean hemoglobin levels (14.1±2.3 g/dL and 14.3±2.2 g/dL; p = 0.25) between the two groups (DR and no DR) in their study. [15]

4.4.6 Hyperlipidemia

There was no significant difference in mean Total Cholesterol, TG, LDL, VLDL and HDL between three groups. We also did not find any statistically significant correlation between LDL and homocysteine levels in either of the three groups in our study.

Similarly Satyanarayana *et al* [15] could not find any significant difference with respect to mean LDL levels between the two groups in their study (DR: 117.1±34 mg/dL vs. No DR: 110.7±29.0 mg/dL; p = 0.6), the mean of both groups was above the upper limit of normal.

4.4.7 Renal dysfunction

There was no significant difference in mean blood urea and serum Creatinine, between three groups. In contrary, there were significantly elevated serum Creatinine levels in the retinopathy group as compared to the no retinopathy group in the study done by Fotiou *et al*.

4.4.8 Homocysteine and retinopathy

Satyanarayana *et al* found significantly higher homocysteine levels in the no retinopathy group as compared to the control group without diabetes. They also showed significantly higher serum homocysteine levels in the patients in the diabetic retinopathy group as compared to the no retinopathy group. Similarly,

Brazionis *et al* showed significantly higher median (IQR) homocysteine level in patients with DR as compared to patients without DR (11.5 µmol/L (10.4– 12.5 µmol/L) vs. 9.6 µmol/L (9.1–10.2 µmol/L), respectively; p=0.001).[16]

Malagaurnera *et al* compared serum homocysteine levels in patients with PDR, NPDR, no DR and healthy controls. There was a significantly higher mean homocysteine level in the PDR group, compared to the no DR group (18.2±5.6 µmol/L vs. 12.1±6.8 µmol/L; p < 0.01). They also found a significant increase in the homocysteine levels with progression of severity of retinopathy. In this study, they found that the odds ratio for hyperhomocysteinemia was 4.2 and 1.2 in the PDR and NPDR groups, respectively.[17]

Hultberg *et al*., in their study, found significantly elevated mean±SD homocysteine level in patients with T1DM with PDR (15.0±6.3µmol/L; p < 0.001) when compared with patients with no or minimal DR (10.7±4.3 µmol/L) or controls (11.0±3.4 µmol/L). They also observed that this increased serum homocysteine level was mainly confined to patients with declining renal function (elevated serum creatinine or deranged albumin: creatinine ratio), and that those with no or minimal renal dysfunction had normal serum homocysteine levels.

Hence, they suggested the role of advanced nephropathy in elevating serum homocysteine levels, rather than diabetes or diabetic retinopathy per se.

In a meta-analysis done by Xu *et al*., including 31 studies and 6394 patients, it was found that the Hcy levels in the blood of patients with DR were higher than that of patients in the control group, although there was statistical heterogeneity among the studies. Xu *et al*. also observed that the role of hyperhomocysteinemia was probably more significant in T1DM or in mixed (Type1+2) diabetes, rather than in patients with T2DM, who constituted our study population.[18]

The mean plasma levels of homocysteine were 15.87 µmol/L in the PDR group and 13.46 µmol/L in the no DR group in the study done by Goldstein *et al*. The authors also found significantly increased homocysteine concentrations with increasing severity of DR. The mean plasma levels in patients with PDR (15.86±1.34 µmol/L) were significantly elevated as compared to levels in patients with NPDR (14.56±0.64 µmol/L), no DR (13.46±0.74

µmol/L), and incontrols (11.75±0.24 µmol/L). [12] Brazionis *et al*, found that, although the levels of homocysteine were significantly higher among the patients with DR, as compared to controls, the difference between the two groups was small (< 2 µmol/L).

Sato *et al*. found that even an increase in 1 µmol/L in homocysteine level was associated with retinopathy, with odds ratio of 1.26, after adjusting for duration of diabetes and glycemic control. Brazionis *et al* also concluded that even a small increase in the serum homocysteine levels, in the order of > 1 µmol/L, can be considered as a useful marker for clinicians to make decisions regarding intensification of treatment of both diabetes and its various comorbidities.

In our study DM group, 30% had increased Serum Homocysteine (>15), in DR group 100% had increased Serum Homocysteine and in Control group 100% had normal Serum Homocysteine. There was significant association between Serum Homocysteine and three groups.

In DM group, mean Homocysteine was 14.90±5.59, in DR group, mean Homocysteine was 38.86±7.69 and in controls, mean Homocysteine was 10.61±1.46. There was significant difference in mean Serum Homocysteine between three groups.

Hence from the study it can be conclude that Retinopathy group had higher Serum Homocysteine than DM and control groups.

	Our study				**Hulter *et al***			
	DM	**DR**	**Control**	**p value**	**DM** (µmol/L)	**DR**	**Control** (µmol/L)	**P value**
Homocysteine (µmol/L)	14.90±5.59	38.86±7.69	10.61±1.46	<0.001	10.7±4.3	15.0±6.3	11.0±3.4	<0.001

	Our study				**Goldstein *et al***			
	DM	**DR**	**Control**	**p value**	**DM** (µmol/L)	**PDR**	**Control**	**P value**
Homocysteine (µmol/L)	14.90±5.59	38.86±7.69	10.61±1.46	<0.001	13.46±0.74	15.86±1.34	11.75±0.24	<0.001

5. Conclusion

1) The prevalence of hyperhomocysteinemia was higher (100%) in the cases with Non proliferative diabetic retinopathy (NPDR), as compared to the Diabetics (30%) and controls (0%). The difference in the prevalence of hyperhomocysteinemia between three was statistically significant (p <0.001*).
2) Mean level of plasmahomocysteine was higher among the DR group (NPDR) as compared to the DM group and controls, the difference was statistically significant.
3) Longer duration of diabetes, and HbA1c which are known risk factors for progression of diabetic retinopathy, were found to be significantly associated with NPDR.

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