

A Study Assessing the Role of Obesity, Smoking and Dyslipidemia in Hyperglycemia Induced Diabetic Retinopathy

¹Dr Sapneh Sharma, Department of Biochemistry, SOS, Jiwaji University, Gwalior, MP

²Dr Poornima Dey Sarkar, Professor and head, Department of Biochemistry, MGM Medical College, Indore, MP

³Dr Arun Mishra, Assistant Professor, Department of Biochemistry, Index Medical College Hospital And Research Center, Indore, MP

Corresponding Author: Dr Arun Mishra, Assistant Professor, Department of Biochemistry, Index Medical College Hospital and Research Center, Indore, MP

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Introduction

Diabetes mellitus (DM) refers to a group of common metabolic irregularities that share the characteristic of hyperglycemia. Various types of diabetes are caused by complex interactions of many factors like genetics, environmental factors, and life-style choices. Depending on the etiology of the diabetes mellitus, reasons leading to hyperglycemia may include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The metabolic irregularities associated with DM causes secondary pathophysiological changes in multiple organ systems that impose a tremendous burden on the individual with diabetes¹. An estimated 439 million people would suffer from type 2 DM by the year 2030².

In India, Type 2 DM is the leading cause of end-stage renal disease (ESRD), non traumatic lower extremity amputations, and adult blindness. With increasing incidences in developing nations like ours, DM will be a leading cause of morbidity and mortality for the foreseeable future. Type 2 DM is due primarily to

lifestyle factors and genetics³. A large number of lifestyle factors are known to be responsible for the development of type 2 DM. These are physical inactivity, sedentary lifestyle, cigarette smoking and generous consumption of alcohol⁴.

Diabetic retinopathy (DR) is a complication of diabetes predominantly affecting the integrity of the microscopic vessels found in the retina. DR can be broadly divided into two stages: non proliferative (NPDR) and proliferative diabetic retinopathy (PDR). DR is a complication of diabetes predominantly affecting the integrity of the microscopic vessels found in the retina. DR can be broadly divided into two stages: non proliferative (NPDR) and proliferative diabetic retinopathy (PDR). During non proliferative stage, the earliest visible sign of retinal damage results from abnormal permeability and/or non perfusion of capillaries, leading to the formation of micro aneurysms. Abnormal capillary permeability results in the leaking of fluid and solutes into the surrounding retinal tissue, which collects around the macula; this is

referred to as macular edema (MO) and it threatens visual acuity. Proliferative stage develops following the occlusion of retinal capillaries leading to retinal ischemia, which promotes the development of neovascularization, a process by which new blood vessels proliferate on the surface of the retina. The newly formed vessels are fragile so they hemorrhage easily. This results in accumulation of blood in the vitreous cavity impairing vision. This may be permanent due to further complications such as traction retinal detachment leading to complete blindness.

Dyslipidemia is one of the major risk factors for both macro and micro vascular disease in diabetes mellitus. The classical features of diabetic dyslipidemia are a low HDL cholesterol concentration, high plasma triglyceride concentration, and increased concentration of small dense LDL-cholesterol particles. The lipid changes associated with diabetes are attributed to increased free fatty acid flux secondary to insulin resistance⁵. Tumor necrosis factor –alpha (TNF-alpha) is considered as one of the potential determinants of insulin resistance⁶. Clinical studies have identified that the elevated serum concentrations of TNF-alpha, and proangiogenic marker vascular endothelial growth factor (VEGF), correlate with the presence and severity of DR⁷.

Material and method

The present research was carried out in the Department of Biochemistry, Mahatma Gandhi Memorial Medical College, Indore. The study included 240 individuals divided in four groups each containing 60 subjects. Group 1 or the control group included healthy subjects (n= 60), group 2 included subjects who had diabetes but no sign of diabetic retinopathy (DWDR, n=60), group 3 included subjects diagnosed with non proliferative diabetic retinopathy (NPDR, n=60), group

4 included subjects with proliferative diabetic retinopathy (PDR, n=60).

HbA1C and fundus dilated examination were the criteria for selection of DR patients. Subjects were screened in the outpatient department and assigned in different groups. The serum sample obtained by processed blood was analyzed for lipid profile parameters and fasting blood glucose estimation using kit methods in auto analyzer (Bioline). Data regarding BMI, age, sex and duration of diabetes type 2 was also recorded. Statistical software, SPSS version 17.0 Trial was used for analysis of data. Overall, selected parametric values among studied samples was recorded, analyzed and compared to establish a relationship between hyperglycemia induced retinopathy, irregular serum lipids and smoking in diabetic subjects. The study was approved by institutional ethical committee and subjects were given a profoma for written consent.

Results

The duration diabetic retinopathy diabetes found to be more among patients of type 2 diabetes mellitus screened positive for proliferative retinopathy as compared to screened negative for non proliferative retinopathy and diabetes mellitus without retinopathy.

Major part of the population (75.0%) of patients of type 2 diabetes mellitus without retinopathy of group 2 were most commonly had diabetic retinopathy duration from 0-5 years as compared to more than one third (38.3%) of patients of group 3 and few (5.0%) patients of group 4 had type 2 diabetes mellitus with retinopathy.

Table 1: Assessment of diabetic retinopathy duration of subjects in groups

Diabetic retinopathy duration	Group 1		Group 2		Group 3		Group 4	
	n ₁	%	n ₂	%	n ₃	%	n ₄	%
0-5 year	0	0.0	45	75.0	23	38.3	3	5.0
5-10 year	0	0.0	15	25.0	28	46.7	32	53.3
10-15 year	0	0.0	0	0.0	8	13.3	17	28.3
≥15 year	0	0.0	0	0.0	1	1.7	8	13.3
Mean ± Std. Deviation	0.0±0.00 year		3.78±1.54 year		6.22±3.34 year		9.72±3.96* year	

*The mean difference in duration is highly significant among four groups at the 0.001 level of significance (p<0.001).

The diabetic retinopathy duration of 5-10 years was observed more common among more than half (53.3%) patients of group 4 had type 2 diabetes mellitus with proliferative retinopathy and that followed by less than half (46.7%) patients of group 3 had type 2 diabetes mellitus with non-proliferative retinopathy and exactly one-fourth (25.0%) patients of group 2 had type 2 diabetes mellitus without retinopathy.

Duration of 10-15 years and ≥15 year was measured only among those patients who had type 2 diabetes mellitus with retinopathy. However, seventeen (28.3%) patients of group 4 had identified with diabetic retinopathy duration of 10-15 years and that followed by eight (13.3%) patients belonged to group 3. The diabetic retinopathy duration of ≥15 year was observed more common among 13.3% patients of group 4 had

type 2 diabetes mellitus with proliferative retinopathy and that followed by one patient of group 3 had type 2 diabetes mellitus with non-proliferative retinopathy. Finally, none of the (0.0%) controls of group 1 had identified with any of the groups of diabetic retinopathy duration.

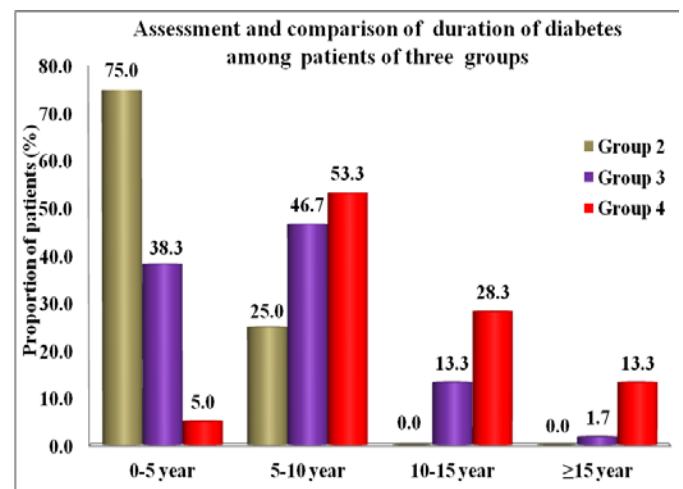


Figure 1: Bar diagram showing the distribution of duration of diabetes of studied patients of type 2 diabetes mellitus of groups 2, 3 and 4.

Table 2: Comparison of total cholesterol, triglyceride, LDL, HDL and VLDL of controls and patients of four groups

Variable	Group	Spread Mean ± SD	95% Confidence Intervals of Mean		p-value (LOS)
			LB	UB	
Total Cholesterol (mg/dl)	Group 1	189.45±14.28	185.76	193.14	F=73.73 p<0.001 [#]
	Group 2	243.30±22.96	237.37	249.23	
	Group 3	230.98±32.96	222.47	239.50	
	Group 4	257.92±31.69	249.73	266.10	
Triglyceride (mg/dl)	Group 1	181.37±20.88	175.97	186.76	F=33.51 p<0.001 [#]
	Group 2	211.02±45.68	199.22	222.82	
	Group 3	223.78±48.67	211.21	236.36	
	Group 4	259.77±51.61	246.43	273.10	
LDL (mg/dl)	Group 1	104.80±17.02	100.41	109.20	F=74.73 p<0.001 [#]
	Group 2	156.20±20.65	150.87	161.54	
	Group 3	145.47±31.31	137.38	153.56	
	Group 4	171.43±30.24	163.62	179.25	
HDL(mg/dl)	Group 1	48.00±10.41	45.31	50.68	F=34.04 p<0.001 [#]
	Group 2	44.96±8.82	42.69	47.24	
	Group 3	41.27±5.37	39.88	42.66	
	Group 4	34.15±6.03	32.59	35.70	
VLDL(mg/dl)	Group 1	36.49±4.25	35.39	37.59	F=33.40 p<0.001 [#]
	Group 2	42.12±9.02	39.79	44.45	
	Group 3	44.83±9.78	42.30	47.35	
	Group 4	52.24±10.62	49.50	54.98	

[#] The mean differences are highly significant at the 0.001 level of significance. [DM-Diabetes Mellitus; non-pro.-non-proliferative; SD-Standard Deviation; LB-Lower Bound, UB- Upper Bound; LOS-Level of Significance]

Statistical analyzes projected that the total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein of diabetic patients with proliferative retinopathy found to be significantly elevated but high density lipoprotein was significantly decreased as compared to patients of diabetes mellitus

with and without retinopathy and healthy controls. However, comparison of lipid profile parameters indicated that the total cholesterol, low and high density lipoproteins of diabetic patients without retinopathy (group 2) found to be elevated than diabetic patients with non-proliferative retinopathy (group 3).

This was observed that the average (mean \pm standard deviation) total cholesterol of diabetes mellitus with proliferative retinopathy patients (257.92 \pm 31.69 mg/dl) found to be significantly larger as compared to diabetes mellitus without retinopathy patients (243.30 \pm 22.96 mg/dl) and that followed by diabetes mellitus patients with non-proliferative retinopathy (230.98 \pm 32.96 mg/dl) and healthy controls (189.45 \pm 14.28 mg/dl). However, one way analysis of variance indicated that these differences in mean total cholesterol among four groups were highly significant ($p < 0.001$).

Among diabetes mellitus with proliferative retinopathy patients, the mean triglyceride (259.77 \pm 51.61 mg/dl) found to be significantly elevated as compared to diabetes mellitus with non-proliferative retinopathy patients (223.78 \pm 48.67 mg/dl) and followed by diabetes mellitus without retinopathy patients (211.02 \pm 45.68 mg/dl) and healthy control patients (181.37 \pm 20.88 mg/dl). However, one way analysis of variance indicated that these differences in mean Triglyceride among four groups were highly significant ($p < 0.001$).

Average LDL of diabetes mellitus with proliferative retinopathy patients (171.43 \pm 30.24 mg/dl) found to be significant increased as compared to diabetes mellitus without retinopathy patients (156.20 \pm 20.65 mg/dl) and that followed by diabetes mellitus with non-proliferative retinopathy patients (145.47 \pm 31.31 mg/dl) and healthy controls (104.80 \pm 17.02 mg/dl). Overall,

one way analysis of variance indicated that these differences in mean LDL among four groups were highly significant ($p < 0.001$).

Average (34.15 \pm 6.03 mg/dl) HDL of diabetes mellitus with proliferative retinopathy patients found to be significantly minimum as compared to diabetes mellitus without retinopathy patients (44.96 \pm 8.82 mg/dl) and that followed by diabetes mellitus with non-proliferative retinopathy patients (41.27 \pm 5.37 mg/dl) and healthy controls (48.00 \pm 10.41 mg/dl). Overall, one way analysis of variance indicated that these differences in mean HDL among four groups were highly significant ($p < 0.001$).

Mean VLDL (52.24 \pm 10.62 mg/dl) levels of diabetes mellitus with proliferative retinopathy patients was significantly elevated as compared to diabetes mellitus with non-proliferative retinopathy patients (44.83 \pm 9.78 mg/dl) and followed by diabetes mellitus without retinopathy patients (42.12 \pm 9.02 mg/dl) and healthy control patients (36.49 \pm 4.25 mg/dl). However, one way analysis of variance indicated that these differences in mean VLDL among four groups were highly significant ($p < 0.001$).

Moreover, the statistical agreement indicated that total cholesterol, triglyceride, LDL and VLDL of non-diabetic and diabetic patients with and without retinopathy found to be significantly differed and noted elevated in diabetic patients with proliferative retinopathy.

Table 3: Distribution and association of body mass index with groups (1 to 4)

Body mass index	Diabetic with or without Retinopathy				Total
	Control Group 1	Group 2	Group 3	Group 4	
<18.5 kg/m ² (Underweight)	-	-	-	-	-
18.5-24.9 kg/m ² (Normal weight)	53 88.3%	10 16.7%	16 26.7%	17 28.3%	96 40.0%
25.0-29.9 kg/m ² (Overweight)	7 11.7%	50 83.3%	38 63.3%	40 66.7%	135 56.3%
≥30.0 kg/m ² (Obese)	0 0.0%	0 0.0%	6 10.0%	3 5.0%	9 3.8%
Total	60 100.0%	60 100.0%	60 100.0%	60 100.0%	240 100.0%

$$\chi^2_6 = 89.64 \text{ and } p < 0.001 \#$$

The association is strongly significant at the 0.001 level of significance.

Statistical analyzes projected that the bonding of body mass index of healthy controls and type 2 diabetes mellitus patients with and without retinopathy found to be significantly strong with groups (group 1, group 2, group 3 and group 4). However, 56.3% samples were most commonly overweight had body mass index between 25.0-29.9 kg/m² while 40.0 cases had normal weight with body mass index between 18.5-24.9 kg/m² but few (3.8%) cases were obese had body mass index of ≥30.0 kg/m² and was measured among all studied (N=240) healthy controls and type 2 diabetes mellitus patients with and without retinopathy. None of the sample was underweight. Analysis of research showed that the body mass index of patients had type 2 diabetes mellitus with and without retinopathy reported more frequently overweight occurred among 50 (83.3%) cases of diabetes mellitus without retinopathy of group 2 and that followed by 40 (66.7%) cases of diabetes

mellitus with proliferative retinopathy of group 4 and among 38 (83.3%) cases of diabetes mellitus with non-proliferative retinopathy of group 3. However, 7 controls had overweight. Normal weight was more frequently noted among 53 (88.3%) controls of group 1 and that followed by 17 (28.3%) cases of group 4 and 16 (26.7%) cases of group 3 and 10 (16.7%) cases of group 2.

Six (10.0%) cases suffered from diabetes mellitus with non-proliferative retinopathy of group 3 cases found to be obese and that followed by 3 (5.0%) diabetes mellitus with retinopathy of group 4 but obesity wasn't identified in rest two groups, 1 and 2.

However, the proportional difference in four groups classified as healthy control (group 1), diabetic without retinopathy (group 2), diabetic with non-proliferative diabetic retinopathy (group 3) and diabetic with proliferative diabetic retinopathy (group 4) with respect to body mass index found to be statistically strongly significant (p<0.0001).

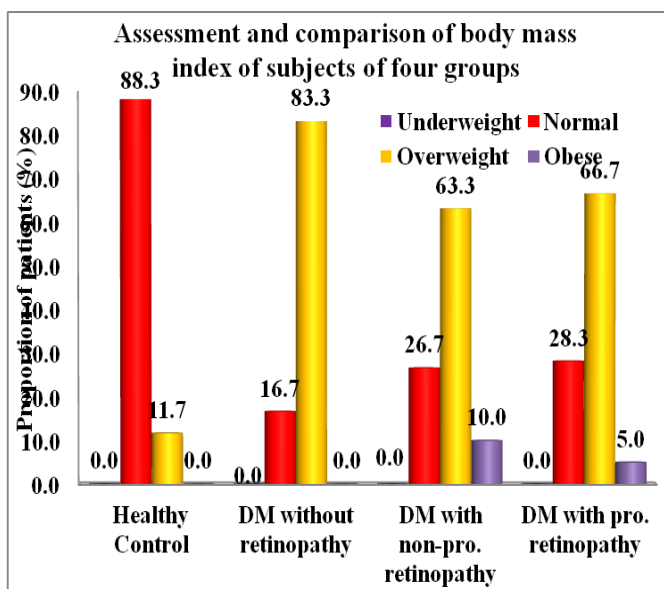


Figure 2: Bar diagram presented the relation of body mass index with four groups of selected healthy controls and type 2 diabetes mellitus patients with and without retinopathy.

Statistical analysis projected that the history of smoking of studied samples wasn't associated significantly with groups (group 1, group 2, group 3 and group 4). However, among all studied (N=240) healthy controls and type 2 diabetes mellitus patients with and without retinopathy more than half (55.8%) of the samples had

not any smoking history but rest (44.2%) observed with history of smoking. Research showed that the smoking history in type 2 diabetes mellitus cases was present more frequently among 31 (51.7%) cases of diabetes mellitus with proliferative retinopathy of group 4 and that followed by 30 (50.0%) cases of diabetes mellitus with non-proliferative retinopathy of group 3, 26 (43.3%) cases of diabetes mellitus without retinopathy of group 3 and 19 (31.7%) healthy controls of group 1.

The smoking history was not recorded among 41 (68.3%) controls of group 1 and that followed by 29 (48.3%), 30(50.0%) and 34(56.7%) cases of group 4, group 3 and group 2 respectively. Nevertheless, the smoking history was not associated significantly ($p>0.05$) with samples of four groups (group 1, group 2, group 3 and group 4). Henceforth, the statistical agreement indicated that the smoking history of patients with diabetes mellitus wasn't the significant factor that impacted the diabetic retinopathy.

Table 4: Distribution and association of smoking history with groups (1 to 4)

History of Smoking	Control	Diabetic with or without Retinopathy			Total
	Group 1	Group 2	Group 3	Group 4	
Absent	41 68.3%	34 56.7%	30 50.0%	29 48.3%	134 55.8%
Present	19 31.7%	26 43.3%	30 50.0%	31 51.7%	106 44.2%
Total	60 100.0%	60 100.0%	60 100.0%	60 100.0%	240 100.0%

$$\chi^2_3 = 6.02 \text{ and } p > 0.05^{\circ}$$

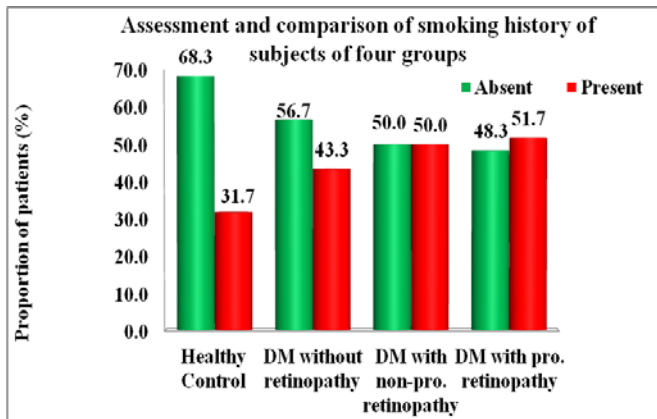


Figure 3 : Bar diagram presented the relation of smoking history with four groups of selected healthy

controls and type 2 diabetes mellitus patients with and without retinopathy.

Table 5: Distribution and association of dyslipidemia with groups (1 to 4)

Dyslipidemia	Control	Diabetic with or without Retinopathy			Total
	Group 1	Group 2	Group 3	Group 4	
Absent	60 100.0%	7 11.7%	10 16.7%	1 1.7%	78 32.5%
Present	0 0.0%	53 88.3%	50 83.3%	59 98.3%	162 67.5%
Total	60 100.0%	60 100.0%	60 100.0%	60 100.0%	240 100.0%

$$\chi^2_3 = 169.35 \text{ and } p < 0.001 \#$$

The association is strongly significant at the 0.001 level of significance.

* The association is significant at the 0.05 level of significance.

Statistical analysis reported that the dyslipidemia of studied samples found to be associated significantly with groups (group 1, group 2, group 3 and group 4). This was identified among all studied (N=240) population that approximately the two-third (67.5%) of the samples diagnosed with dyslipidemia but rest (32.5%) didn't diagnosed with dyslipidemia.

Research showed that the dyslipidemia was diagnosed more frequently among type 2 diabetes mellitus cases was noted among approximately all (98.3%) cases of

diabetes mellitus with proliferative retinopathy of group 4 and that followed by 53 (88.3%) cases of diabetes mellitus without retinopathy of group 2, 50 (83.3%) cases of diabetes mellitus with non-proliferative retinopathy of group 3. But dyslipidemia was not diagnosed in any of (0.0%) the healthy controls of group 1.

The dyslipidemia wasn't diagnosed among 10 (16.7%), 7 (11.7%) and 1 (1.7%) cases of group 3, group 2 and group 4 respectively. Overall, the dyslipidemia found to be the strongly associated factor that significantly ($p < 0.0001$) influenced the proportional differences in samples of four groups (group 1, group 2, group 3 and group 4).

Moreover, the statistical agreement indicated that the dyslipidemia of patients with diabetes mellitus was the highly significant factor that impacted the diabetic retinopathy.

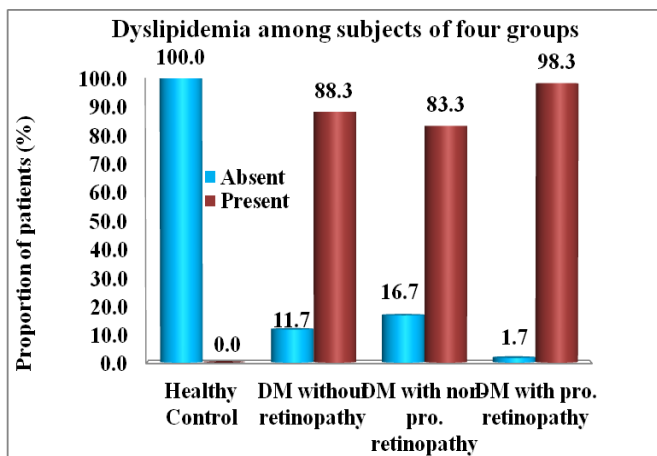


Figure 4: Bar diagram presented the association of dyslipidemia with samples of four groups

Discussion

Duration of diabetes is an important factor in the development of its complications. The incidence of DR increases with diabetes duration, increasing linearly after 10 years, and it is detected in one-third of all patients with DM for 25 years⁸. In our study we found that the major part of the population (75.0%) of patients of group 2 (DWDR) who didn't had retinopathy had 0-5 years of duration of diabetes.

The duration of 5-10 years was observed more common among patients of group 4 (53.3%) with proliferative retinopathy (PDR) followed by less than half (46.7%) patients of group 3 (NPDR) and exactly one-fourth (25.0%) patients of group 2(DWDR). Diabetes duration of 10-15 years was seen only among group 3 and group 4. The diabetes duration of ≥ 15 years was observed more common among 13.3% patients of group 4 with proliferative retinopathy followed by 1.7% of group 3 patients. Our results clearly suggest that as the duration of DM progresses the risk of retinopathy also increases. An updated report of the landmark Wisconsin

Epidemiologic Study of Diabetic Retinopathy found that the predominant risk factors for proliferative DR were duration of diabetes and glycemic control, and that risk may be further increased as the duration of diabetes increases⁹.

In the study it was observed that the average (mean \pm standard deviation) fasting blood sugar (FBS) of patients of diabetes mellitus with proliferative retinopathy (PDR) (192.43 ± 30.72 mg/dl) was found to be significantly higher as compared with non-proliferative retinopathy (NPDR) (180.58 ± 14.49 mg/dl) and that followed by diabetes mellitus without retinopathy patients (166.65 ± 21.12 mg/dl) and healthy controls (110.35 ± 11.54 mg/dl). However, one way analysis of variance indicated that these differences in mean FBS among four groups were highly significant ($p < 0.001$).

HbA1c levels, which can reflect a person's average level of blood glucose over the past three months can impact a number of molecular and cellular processes that may influence retinopathy development and severity, including microvasculature complication, ischemia, increased levels of vascular endothelial growth factor (VEGF) and inflammation^{10,11}. Among patients of diabetes mellitus with proliferative retinopathy patients, the mean Hb1Ac (9.26 ± 0.84 %) found to be significantly elevated as compared to diabetes mellitus with non-proliferative retinopathy patients (8.16 ± 0.61 %) and followed by diabetes mellitus without retinopathy patients (7.41 ± 0.59 %) and healthy controls (5.83 ± 0.63 %). Also one way analysis of variance (ANOVA) indicated that these differences in mean Hb1Ac among four groups were highly significant ($p < 0.001$).

The WESDR XIII study there was a significant trend towards an association between increasing severity of

diabetic retinopathy and of retinal hard exudate and increasing cholesterol in diabetic persons¹². Statistical analysis of our study has projected that total cholesterol, triglyceride, low density lipoprotein and very low density lipoprotein of diabetic patients with proliferative retinopathy found to be significantly elevated but high density lipoprotein was significantly decreased as compared to patients of diabetes mellitus with and without retinopathy and healthy controls.

According to Hoorn study (2002) the patients who had elevated serum total cholesterol or elevated serum low-density lipoprotein cholesterol (LDL-C) were more likely to have retinal hard exudate. The prevalence of retinopathy was positively associated with elevated BMI, serum cholesterol and triglyceride levels in all glucose categories. In addition, elevated blood pressure and plasma total and LDL cholesterol levels showed associations with retinal hard exudate¹³. In our study it was observed that the average (mean \pm standard deviation) total cholesterol of diabetes mellitus with proliferative retinopathy patients (257.92 \pm 31.69 mg/dl) was found to be significantly larger as compared to diabetes mellitus without retinopathy patients (243.30 \pm 22.96 mg/dl) and that followed by diabetes mellitus patients with non-proliferative retinopathy (230.98 \pm 32.96 mg/dl) and healthy controls (189.45 \pm 14.28 mg/dl). However, one way analysis of variance indicated that these differences in mean total cholesterol among four groups were highly significant ($p < 0.001$).

Among diabetes mellitus with proliferative retinopathy patients, the mean triglyceride (259.77 \pm 51.61 mg/dl) was found to be significantly elevated as compared to diabetes mellitus with non-proliferative retinopathy patients (223.78 \pm 48.67 mg/dl) and followed by diabetes mellitus without retinopathy patients (211.02 \pm 45.68

mg/dl) and healthy control patients (181.37 \pm 20.88 mg/dl). However, one way analysis of variance indicated that these differences in mean Triglyceride among four groups were highly significant ($p < 0.001$).

Average LDL of diabetes mellitus with proliferative retinopathy patients (171.43 \pm 30.24 mg/dl) was found to be significant increased as compared to diabetes mellitus without retinopathy patients (156.20 \pm 20.65 mg/dl) and that followed by diabetes mellitus with non-proliferative retinopathy patients (145.47 \pm 31.31 mg/dl) and healthy controls (104.80 \pm 17.02 mg/dl). Overall, one way analysis of variance indicated that these differences in mean LDL among four groups were highly significant ($p < 0.001$). The mean (34.15 \pm 6.03 mg/dl) HDL of diabetes mellitus with proliferative retinopathy patients was found to be significantly minimum as compared to diabetes mellitus without retinopathy patients (44.96 \pm 8.82 mg/dl) and that followed by diabetes mellitus with non-proliferative retinopathy patients (41.27 \pm 5.37 mg/dl) and healthy controls (48.00 \pm 10.41 mg/dl). Overall, one way analysis of variance indicated that these differences in mean HDL among four groups were highly significant ($p < 0.001$).

Our study indicated that total cholesterol, triglyceride, LDL and VLDL of non-diabetic and diabetic patients with and without retinopathy found to be significantly differed and noted elevated in diabetic patients with proliferative retinopathy

Although an increasing number of epidemiologic studies have been performed to analyze the association between BMI and DR, the results are still inclusive. Most studies have reported a significant association between high BMI and obesity with DR¹⁴. The result of our study is consistent with these findings. Analysis of research showed that the body mass index of patients

had type 2 diabetes mellitus with and without retinopathy reported more frequently overweight occurred among 50 (83.3%) cases of diabetes mellitus without retinopathy of group 2 and that followed by 40 (66.7%) cases of diabetes mellitus with proliferative retinopathy of group 4 and among 38 (83.3%) cases of diabetes mellitus with non-proliferative retinopathy of group 3. However, 7 controls were overweight. Normal BMI was more frequently noted among 53 (88.3%) controls of group 1 and that followed by 17 (28.3%) cases of group 4 and 16 (26.7%) cases of group 3 and 10 (16.7%) cases of group 2. 10.0% cases group 3 were found to be obese followed by 5.0% of group 4 but obesity wasn't identified in rest two groups, 1 and 2. However, the proportional difference in four groups classified as healthy control (group 1), diabetic without retinopathy (group 2), diabetic with non-proliferative diabetic retinopathy (group 3) and diabetic with proliferative diabetic retinopathy (group 4) with respect to body mass index found to be statistically strongly significant ($p < 0.0001$).

The history of smoking was not associated significantly ($p > 0.05$) with samples of four groups (group 1, group 2, group 3 and group 4). Earlier studies have indicated that the smoking history of patients with diabetes mellitus wasn't the significant factor that impacted the diabetic retinopathy¹⁵. As far results of our study are concerned smoking is not likely to be an important risk factor for diabetic retinopathy.

Conclusion

Our study statically weighs that as the duration of diabetes increases the risk of advanced stage of retinopathy also increases. Obesity, high BMI and dyslipidemia lead to development of different stages of diabetic retinopathy. Some sedentary lifestyle choices

(smoking and obesity) might have their role in microvascular complication of diabetic retinopathy.

Authors Contribution

All Authors contributed equally in this study.

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