Original article

Association between diabetic retinopathy and serum lipoproteins level

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Abstract

Introduction: Diabetic retinopathy is the leading cause of blindness of working-age patients in the urban areas.

Objective: To find out the association between diabetic retinopathy and serum lipoproteins in patients with diabetes mellitus

Materials and methods: A community-based cross-sectional study involving 100 subjects with type II diabetes living in the cities of Kathmandu, Bhaktapur and Lalitpur of Nepal was carried out using a purposive sampling method. The retinopathy was classified according to the modified Airlie House classification of diabetic retinopathy. The patients with systolic hypertension were excluded. The blood sugar level was assessed by using glycosylated hemoglobin. The fasting serum lipoproteins were assessed by photometric enzymatic methods.

Statistics: The SPSS version 10.0 software was used. The p value of <0.05 was considered significant.

Results: The majority (n=91) of the patients were in the age group 31-60 years with the mean of 48.2 ± 9.5. The mean duration of diabetes mellitus in the patients with no-diabetic-retinopathy was 4 ±3.4 years and that in those with the retinopathy was 6.4 ± 4years, (p =0.006). The mean glycosylated hemoglobin level for no-diabetic-retinopathy group was 6.9 ±1.1 %, whereas for the diabetic retinopathy group, it was 7.7 ± 1.5% (p = 0.004). The mean values of serum lipoproteins when taken together were slightly higher in the diabetic retinopathy group than in the group with no-diabetic-retinopathy. The serum triglyceride value was higher in the group with diabetic retinopathy than in the group with no retinopathy. Serum triglyceride had a low degree of positive correlation with HbA1c value.

Conclusion: There is no significant association between the serum lipoprotein levels and diabetic retinopathy in patients with type II diabetes mellitus. Serum triglyceride level has a low degree of positive correlation with HbA1c value.

Keywords: diabetic retinopathy, lipoproteins, glycosylated hemoglobin

Introduction

Diabetes mellitus is a metabolic disease caused by an absolute or relative insufficiency of insulin. Long-term hyperglycemia is associated with changes in the microvasculature including kidney, heart, retina and other parts of the body. There is now ample evidence to show that the development of diabetic retinopathy is a multi-factorial process where genetic, metabolic and growth factors play an important role (Giusti C & Gargiulo P, 2007). Further identification of risk factors and determinants for retinopathy is important to improve understanding of the disease mechanisms and to facilitate new treatments and preventive strategies. There are reports suggesting serum Lp(a) level as an independent risk factor for the progression of non-proliferative diabetic retinopathy (NPDR) in type 2 diabetes patients. It has, however, been recommended by some researchers that such findings be validated (Funatsu H et al 2009).

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Lipoprotein is atherogenic and thrombogenic in nature. Its oxidative form causes injury to retinal capillaries causing retinopathy. The present study was undertaken to evaluate the association between the diabetic retinopathy and serum lipoprotein level at first presentation in type II diabetic patients of an urban population of the Kathmandu valley.

Materials and methods

A cross-sectional study was carried out involving 100 subjects with type II diabetes using a purposive-sampling method. All type 2 diabetic patients screened at a diabetes education camp conducted by Astha Nepal in the urban Kathmandu valley were advised to attend the BP Koirala Lions Centre for Ophthalmic Studies (BPKLCOS) for detailed evaluation.

The patients with type 1 diabetes, hypertension, post cataract/ vitrectomy surgery, pregnancy, hazy media, retinal vaso-occlusive disease and renal diseases were excluded.

A pro forma was the tool for data collection. A dilated posterior segment examination was done with combination of 0.8% tropicamide and 5% phenyliphrine eye drops.

The Airlie House Classification of diabetic retinopathy which was subsequently modified by the Diabetic Retinopathy Study and extended by the Early Treatment Diabetic Retinopathy Study was followed.

Measurement of Hba1c level and serum lipoproteins

Glycosylated hemoglobin level and fasting serum lipoproteins of all the patients were measured. HbA1c level was recorded by gel precipitation method and was graded as follows.

- Normal range : 4.2-6.2%
- Good control : 6.3%-6.8%
- Fair control : 6.9-7.6%
- Poor control : >7.6%

Serum lipoproteins	Normal range
Triglyceride	0.5-1.8mmol/l
Cholesterol	3.5-8.5mmol/l
HDL*	0.8-1.6mmol/l
LDL*	Upto 4.0mmol/l

*HDL=high density lipoprotein, LDL = low density lipoprotein

Measurement of serum lipoproteins was done using photometric enzymatic methods. The following levels were considered normal.

Statistics

The SPSS version 10.0 was used for data analysis. A value of $p<0.05$ was considered significant. A statistician was consulted as and when necessary.

Ethical issues

An informed consent was obtained from the patients.

Results

In this study, 100 subjects (200 eyes) with type 2 diabetes mellitus (DM) were enrolled. The majority (91%) of diabetics were in the working-age groups of 31-60 years. The mean age was 48.2 ± 9.5 years (Table 1). Diabetes was found mostly in housewives followed by service holders (Table 2). The mean duration of diabetes was 4.5 ± 3.6 years. 74% of the patients were on oral hypoglycemic drugs, 4% were on insulin and the rest on dietary control. The glycosylated hemoglobin had similar distribution in all levels. Mean glycosylated hemoglobin level was 7.0 ± 1.2 %.

Table 1

Age distribution

Age (in years)	No. of subjects	Percentage
21-30	2	2
31-40	18	18
41-50	37	37
51-60	36	36
61-70	7	7
Total	100	100

Table 2

Occupation distribution

Occupation	Frequency	Percentage
Housewife	28	28
Service	27	27
Business	24	24
Teacher	9	9
Retired	4	4
Farmer	3	3
Others	5	5
Total	100	100

Table 3
Duration of diabetes mellitus

Duration of diabetes mellitus (years)	No. of patients	Percent
0-2	39	39
>2-4	14	14
>4-6	14	14
>6-8	13	13
>8-10	15	15
>10-12	5	5
Total	100	100

Table 4
Glycosylated Hb level

Glycosylated Hb level	No. of patients	%
Normal (4.2 to 6.2 %)	26	26
Good (>6.2 to 6.8%)	27	27
Fair (>6.8 to7.6%)	21	21
Poor (>7.6%)	26	26
Total	100	100

Table 5
Grading of diabetic retinopathy (DR) in both eyes

Stage of diabetic retinopathy	Right eye	Left eye
No retinopathy	79(79%)	79(79%)
Mild NPDR	17(17%)	17(17%)
Moderate NPDR	1(1%)	1(1%)
Severe NPDR	3(3%)	3(3%)
PDR	0(0%)	0(0%)
Total	100	100

21% of patients had diabetic retinopathy at first presentation. Among them, the highest number of patients had mild NPDR (17%), followed by severe NPDR (3%) and moderate NPDR (1%).

The severity of diabetic retinopathy was found to be increasing with the duration of diabetes. In the right eyes of the patients with duration of diabetes up to 2 years, there was no diabetic retinopathy in 87.2%, whereas mild diabetic retinopathy was found in 10.3% and moderate diabetic retinopathy in 2.6%. A 10 -12 years of duration was associated with increased number and severity of diabetic retinopathy.

The mean duration of diabetes for no-diabetic retinopathy group was 4±3.4 years and for diabetic retinopathy group, it was 6.4 ±4 years. The duration of diabetes was significantly longer in the diabetic retinopathy group than in the no-diabetic-retinopathy group (p: 0.006). In the normal-glycosylated-Hb group, 92.3% had no diabetic retinopathy in the right eyes. Likewise, in normal-glycosylated-Hb group, 88.5% had no diabetic retinopathy in the left eye.

Table 6
Relationship of stage of retinopathy and level of HbA1c for the right eye

Stage of diabetic retinopathy (RE)	Status of glycosylated Hb and number of eyes (%)			
	Nor-mal	Good control	Fair control	Poor control
No DR	24 (92.3%)	23 (85.2%)	16 (76.2%)	16 (61.5%)
Mild NPDR	1 (3.8%)	4 (14.8%)	4 (19.0%)	8 (30.8 %)
Moderate NPDR	0	0	1 (4.8%)	0
Severe NPDR	1 (3.8%)	0	0	2 (7.7%)
Total	26 (100%)	27 (100%)	21 (100%)	26 (100%)
No. of eyes with retinopathy (%)	2 (7.6%)	4 (14.8%)	5 (23.8%)	10 (38.5%)

Table 7
Relationship of stage of retinopathy and level of HbA1c for the left eye

Stage of diabetic retinopathy (LE)	Status of glycosylated Hb and number of eyes (%)			
	Normal	Good control	Fair control	Poor control
No DR	23 88.5%	23 85.2%	15 71.4%	18 69.2%
Mild NPDR	2 7.7%	4 14.8%	6 28.6%	5 19.2%
Moderate NPDR	0	0	0	1 3.8%
Severe NPDR	1 3.8%	0	0	2 7.7%
Total	26	27	21	26
No. of eyes with retinopathy (%)	11.5% (3)	14.8% (4)	28.8% (6)	30.8% (8)

The mean glycosylated hemoglobin level was 7.0 ± 1.2 (%). The mean glycosylated hemoglobin level (%) for the no-diabetic-retinopathy group was 6.9±1.1 and for the diabetic-retinopathy group, it was 7.7±1.5%. The HbA1c value was significantly higher in the diabetic retinopathy group than in the no- diabetic retinopathy group (p = 0.004). In up to 2 years of duration of DM, no diabetic retinopathy was found in the normal-HbA1c group. In up to 2 years of duration of diabetes, no diabetic retinopathy was found in normal HbA1c group and the good-HbA1c group.

Table 8
Relationship between duration of diabetes, level of glycosylated hemoglobin and stage of diabetic retinopathy in the right eye (RE)

Duration of diabetes (years)	HbA1c	Stage of diabetic retinopathy (RE)				Total	% of retinopathy (n)
		No DR	Mild NPDR	Mod. NPDR	Severe NPDR		
0-2	Normal	12				12	12.9%
	Good	12	1			13	(5)
	Fair	7		1		8	
	Poor	3	3			6	
	Total	34	4	1		39	
2.1-4	Normal	4				4	14.3%
	Good	4				4	(2)
	Fair	2	2			4	
	Poor	2				2	
	Total	12	2			14	
4.1-6	Normal	2				2	28.6%
	Good	3	1			4	(4)
	Fair	1	1			2	
	Poor	4	2			6	
	Total	10	4			14	
6.1-8	Normal	4				4	15.4%
	Good	2				2	(2)
	Fair	4				4	
	Poor	1	1		1	3	
	Total	11	1		1	13	
8.1-10	Normal	2	1			3	33.3%
	Good	2	1			3	(5)
	Fair	2	1			3	
	Poor	4	2			6	
	Total	10	5			15	
10.1-12	Normal				1	1	60%
	Good		1			1	(3)
	Fair						
	Poor	2			1	3	
	Total	2	1		2	5	

Table 9
Relationship between duration of diabetes, level of glycosylated hemoglobin and stage of diabetic retinopathy in the left eye (LE)

Duration of diabetes (YEARS)	HbA1c	Stage of diabetic retinopathy (LE)				Total	% of retinopathy
		Normal	Mild NPDR	Mod. NPDR	Severe NPDR		
0-2	Normal	12				12	7.7%
	Good	13				13	(3)
	Fair	6	2			8	
	Poor	5	1			6	
	Total	36	3			39	
2.1-4	Normal	4				4	14.3%
	Good	4				4	(2)
	Fair	2	2			4	
	Poor	2				2	
	Total	12	2			14	
4.1-6	Normal	2				2	21.4%
	Good	3	1			4	(3)
	Fair	2				2	
	Poor	4	1	1		6	
	Total	11	2	1		14	
6.1-8	Normal	2	2			4	47.2%
	Good	1	1			2	(6)
	Fair	3	1			4	
	Poor	1	1		1	3	
	Total	7	5		1	13	
8.1-10	Normal	3				3	26.7%
	Good	2	1			3	(4)
	Fair	2	1			3	
	Poor	4	2			6	
	Total	11	4			15	
10.1-12	Normal				1	1	60%
	Good		1			1	(3)
	Fair						
	Poor	2			1	3	
	Total	2	1		2	5	

Table 10
Association between diabetic retinopathy and serum lipoproteins

No DRP (n=79) DRP (n=21)

Variables	NO DRP		DRP		p
	mean	±SD	mean	±SD	value
LDL	3.77	0.83	3.87	0.64	0.63
Triglyceride	2.42	0.64	2.53	0.96	0.56
HDL	1.26	0.31	1.25	0.25	0.88
Cholesterol	5.59	0.89	5.65	0.60	0.75
HbA1c	6.9%	1.1%	7.7%	1.5%	0.004

The mean values of serum lipoproteins were higher in the diabetic-retinopathy group than in the no-diabetic-retinopathy group. But the difference was not statistically significant (Table 10). In the normal-HbA1c and the no-retinopathy group, 62.5% had normal LDL level and 37% had high LDL level. In the normal-HbA1c and the no-retinopathy group, 16.6% had normal triglyceride level and 83.4% had high triglyceride level,

whereas in the normal-HbA1c and retinopathy group, high triglyceride level was found in all of the patients (Table 11). All 4 serum lipoproteins had low degree of positive correlation with HbA1c value (Table 12).

All serum lipoproteins had low degree of negative correlation with HbA1c value except serum triglyceride which had low degree of positive correlation with HbA1c value (Table 13). All serum lipoproteins had low degree of negative correlation with HbA1c value except serum triglyceride which had low degree of positive correlation with HbA1c value.

All serum lipoproteins had low degree of positive correlation with HbA1c value, except serum HDL, which had a low degree of negative correlation with HbA1c value (Table 14).

Table 11
Relationship between serum lipoproteins, level of glycosylated hemoglobin and diabetic retinopathy

Serum lipoproteins	HbA1c	Diabetic retinopathy	Serum lipoproteins		Total	p value
			Normal	High		
Serum LDL	Normal	No DRP	15	9	24	1.00
			62.5%	37.5%	100.0%	
		DRP	1	1	2	
			50%	50%	100.0%	
		Total	16	10	26	
	Abnormal	No DRP	32	23	55	1.00
			58.2%	41.8%	100.0%	
		DRP	11	8	19	
			57.9%	42.1%	100.0%	
		Total	43	31	74	
Serum Triglyceride	Normal	No DRP	4	20	24	1.00
			16.6%	83.4%	100.0%	
		DRP	0	2	2	
				100.0%	100.0%	
		Total	4	22	26	
	Abnormal	No DRP	12	43	55	0.74
			21.8%	78.2%	100.0%	
		DRP	3	16	19	
			15.7%	84.3%	100.0%	
		Total	15	59	74	
Serum HDL	Normal	No DRP	24	0		0.045
			100%		100%	
		DRP	2	0		
			100%		100%	
		Total	26	0		
	Abnormal	No DRP	54	1	55	1.00
			98.1%	1.9%	100%	
		DRP	19	0	19	
			100%		100%	
		Total	73	1	74	
Serum Cholesterol	Normal	No DRP	24	0	24	0.82
			100%		100%	
		DRP	2	0	2	
			100%		100%	
		Total	100%	100%	100%	
	Abnormal	No DRP	55	0	0	0.88
			100%			
		DRP	19	0	0	
			100%			
		Total	100%	0	0	

Table 12
Correlation between normal HbA1c and serum lipoproteins in subjects with no DR (n=24)

Lipoproteins	Karl-Pearson correlation coefficient r	P value
LDL	0.2	0.3
Triglyceride	0.4	0.05
HDL	0.14	0.5
Cholesterol	0.36	0.07

Table 13
Correlation between abnormal HbA1c and serum lipoproteins in subjects with no DR (n=55)

Correlation betn HbA1c & serum lipoproteins	Karl Pearson correlation coefficient r	p value
LDL	--0.13	0.33
Triglyceride	0.11	0.4
HDL	-0.13	0.33
Cholesterol	-0.2	0.13

Table 14
Correlation between abnormal HbA1c and serum lipoproteins in subjects with DR (n=19)

Serum Lipoproteins	Karl Pearson correlation coefficient r	p value
LDL	0.36	0.12
Triglyceride	0.15	0.54
HDL	-0.08	0.72
Cholesterol	0.09	0.7

Discussion

One hundred subjects (200 eyes) with type II diabetes were included in this study. The age of them ranged from 21 to 70 years with a higher number of diabetics in the working-age groups 31-61 years (91%). Mean age was 48.2 ± 9.5 years. In the developed world, the majority of diabetics are aged 65 years and above, whereas in the developing world, the majority of diabetics are in the age group of 45-64 years. According to the Chennai Urban Rural Epidemiology Study (CURES) Eye Study I, the prevalence of diabetes in the 45 to 60 years-age-group was nearly 25% (Rema M et al 2005). In a study done in 1980 in the west of Scotland, diabetes was responsible for 10% of blindness and was the commonest cause of blindness in the age groups of 45-54 and 55-64 years (Ghafour I et al 1983).

In this study, the female to male ratio of diabetics was 1:1.77 with a higher prevalence of the disease in males. In another study done in a department of ophthalmology in Kathmandu the female to male ratio of diabetics was 1:1.2 (Karki M, 2004; personal communication, unpublished). Diabetes was found mostly in housewives (28%).

In this study, the patients were screened from the urban diabetic community education camp. The duration of diabetes was significantly longer in the diabetic retinopathy group than in the no-diabetic-retinopathy group (p=0.006). 60% of diabetics of 10-12 year-duration had diabetic retinopathy, whereas 7.7% - 12.9% of diabetics of 0 to 2 year- duration had diabetic retinopathy. Michael Colucciello found that more than 80% of patients who have had diabetes for 15 years also have evidence of retinopathy, the hallmark of which is retinal capillary microaneurysms (Colucciello M, 2004). Among younger-onset patients with diabetes in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), the prevalence of diabetic retinopathy was 8% at 3 years, 25% at 5 years, 60% at 10 years, and 80% at 15 years. The prevalence of PDR was 0% at 3 years and increased to 25% at 15 years (Klein R et al 1984).

This study showed that 21% of patients had diabetic retinopathy. Among them, the highest number of patients had mild NPDR (17%) followed by severe NPDR (3%) and moderate NPDR (1%). Similarly, Fong et al also found that up to 21% of patients with type 2 diabetes had retinopathy at the time of first diagnosis of diabetes, and most developed some degree of retinopathy over time (Donald S. et al 2003). In the study of Chennai Urban Rural Epidemiology Eye Study (CURES) I, the overall prevalence of diabetic retinopathy in the population was 17.6% (95% CI = 15.8–19.5), which included 20.8% (95% CI: 18.7–23.1) in known diabetic subjects and 5.1% (95% CI: 3.1–8.0) in subjects with newly detected diabetes (Rema et al 2005).

In this study, the mean glycosylated hemoglobin level was 7.0 ± 1.2 %. The mean glycosylated hemoglobin for the no-diabetic retinopathy group was 6.9±1.1% and for the diabetic retinopathy group, it was 7.7±1.5%. The HbA1c value was significantly higher in the diabetic retinopathy group than in the no-diabetic-retinopathy group (p = 0.004). In a South East Asian study, the median HbA1c level was 7.5% (Raheja et al 2001). Salem et al studied diabetic retinopathy among Jordanians and found that mean HbA1c was 7.9±1.63 % in normal retina and 9.1%±2.18 (p =0.002) in diabetic retinopathy (Mahmoud et al 1999).

In this study, 30.8% - 38.5% of diabetics of the poor-HbA1c group had diabetic retinopathy and 7.6% - 11.5% of diabetics of the normal HbA1c group had diabetic retinopathy. The CURES Eye Study I found for every 2% elevation of HbA1c, the risk for diabetic retinopathy increased by a factor of 1.7. For every 2% increase in HbA1c, the risk for diabetic retinopathy was higher in those with longer duration of diabetes (Rema M et al 2005).

In this study, all 4 serum lipoproteins (LDL, Triglyceride, HDL and total cholesterol) were measured. The mean lipoprotein values were slightly higher in the diabetic retinopathy group than in the no-diabetic retinopathy group. They were, however, not statistically significant. Serum triglyceride was high in the normal HbA1c and abnormal HbA1c groups and it was higher in the diabetic retinopathy group than in the no-diabetic-retinopathy group. Serum triglyceride had a low degree of positive correlation with HbA1c value. A study conducted by the DCCT/EDIC Research Group (1993) found that ETDRS scores were significantly related to the triglyceride level in univariate analysis, whereas multivariate analysis exhibited only a borderline association in the combined cohort only and LDL cholesterol tended to increase in both genders with more

severe retinopathy, but this was not significant in the multivariate analysis. HDL cholesterol was inversely associated with ETDRS scores in both genders with univariate analysis. More severe retinopathy was associated with a higher total triglyceride level and a lower HDL cholesterol level. Retinopathy was strongly associated with small and medium, but not large VLDL in each gender. A similar study done in Turkey by Ergun et al (2004) on lipoprotein (A) levels in type 2 diabetic patients with diabetic retinopathy revealed that the Lp(a) levels were similar in the patients with retinopathy and those without retinopathy. There was no evidence for a relationship between the serum Lp(a) levels and diabetic retinopathy in type 2 diabetic patients (Ergun UG et al 2004).

Conclusion

There appears to be no statistically significant relationship between diabetic retinopathy and serum lipoproteins. The serum lipoprotein levels are slightly higher in the patients with diabetic retinopathy than in those without retinopathy.

All 4 serum lipoproteins have low degree of positive correlation with HbA1c value demonstrating some role of lipoproteins in pathogenesis of diabetic retinopathy.

References

American Diabetes Association (2003). Treatment oh Hypertension in adults with diabetes. Diabetes Care. 26(suppl); S80-s82.

Colucciello M (2004). Diabetic retinopathy: control of systemic factors preserves vision. Postgraduate Medicine; 116: 57-64.

Diabetes Control and Complications Trail Research Group (1993). The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med; 329:977-986.

Diabetes Control and Complications Trail Research Group (1995). Progression of retinopathy with intensive versus conventional treatment in Diabetes Control and Complications Trail.Ophthalmology; 102:647-661.

Donald S. Fong, Lloyd Aiello et al (2003). Diabetic Retinopathy by American Diabetes Association : Diabetes Care 26:S99-S102,.

Ergun UG, Oztuzun S, Seydaoglu G (2004): Lipoprotein (A) levels in type 2 diabetic patients with diabetic retinopathy Med J Malaysia;59(3):406-10.

Funatsu H, Shimizu E, Noma H, Mimura T, Hori S (2009). Association between serum lipoprotein (a) level and progression of non-proliferative diabetic retinopathy in Type 2 diabetes. Acta Ophthalmol. 87(5):501-5.

Ghafour I, Alan D, Foulds W (1983). Common causes of blindness and visual handicap in the west of Scotland. Br J Ophthalmol; 67:209-213.

Giusti C, Gargiulo P (2007). Advances in biochemical mechanisms of diabetic retinopathy. Eur Rev Med Pharmacol Sci.; 11(3):155-63.

Klein R, Klein BE, Moss SE, Davis MD, DeMets DL (1984). The Wisconsin Epidemiologic Study of Diabetic Retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years. Arch Ophthalmol 102:520–526.

Mahmoud S et al (1999). Diabetic retinopathy among Jordanians: its pattern, severity, and some associated risk factors. Diabetologica Croatica; National Center for Diabetes, Endocrine and Hereditary Diseases, Amman, Jordan. http://www.idb.hr/diabetologia/99no1-2.html. Accessed on 09.08.2009 for verification.

Raheja BS, Kapur A, Bhoraskar A, Sathe SR, Jorgensen LN, Moorthi SR et al (2001). Diab Care Asia—India Study: diabetes care in India-current status. J Assoc Physicians India; 49:717-22.

Rema M, Premkumar S et al (2005). Prevalence of Diabetic Retinopathy in Urban India: The Chennai Urban Rural Epidemiology Study (CURES) Eye Study, I. Investigative Ophthalmology and Visual Science; 46:2328-2333.

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