Original Article

Association of Serum Lipids Levels in Patients of Type 2 Diabetic Retinopathy and Without Retinopathy

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Abstract

Background: Diabetic retinopathy is a major micro-vascular complication of diabetes accounting for its leading cause of irreversible blindness worldwide. Assessing the risk factors of diabetic retinopathy, particularly modified risk factors, is important for early intervention to reduce the onset and progression of diabetic retinopathy. **Subjects and Methods:** Twenty eight Patients with newly diagnosed type 2 diabetes mellitus without any signs of retinopathy were identified from outpatient services of department of Medicine and Twenty eight Patients with newly diagnosed type 2 diabetes mellitus with retinopathy were identified from outpatient services of department of Ophthalmology along with Twenty eight healthy subjects were taken as controls. **Results:** Increased levels of Triglycerides and High density lipoprotein cholesterol were observed in both set of patients with newly diagnosed type 2 diabetes mellitus (with or without diabetic retinopathy) as compared to the healthy subjects which were statistically significant. **Conclusion:** Triglycerides and high density lipoprotein cholesterol is associated with the increased incidence in diabetes mellitus subjects.

Keywords: Diabetic retinopathy, fasting blood sugar, HbA1c and lipid profile.

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Introduction

India is going to become the diabetic capital of the world. According to the WHO, 72 million people were affected by diabetes mellitus in India in the year 2017, a figure expected to almost double to 134 million by 2025, the largest number in any country in the world. Almost two third of all Type 2 and almost all Type 1 diabetics are expected to develop diabetic retinopathy over a period of time.[1,2] Diabetic retinopathy is a major micro-vascular complication of diabetes accounting for its leading cause of irreversible blindness worldwide. Assessing the risk factors of diabetic retinopathy, particularly modified risk factors, is important for early intervention to reduce the onset and progression of diabetic retinopathy. Several population-based epidemiological studies have investigated the risk factors of diabetic retinopathy.[3] Visual disability associated with diabetes mellitus is a significant public health problem. Diabetic retinopathy is one such visual disability which has got significant adverse effect on quality of life of these patients. Pathogenesis of diabetic retinopathy is multifactorial and various hypotheses have been proposed. Despite the fact that this complication of diabetes mellitus is largely preventable, no definitive evidence has been proposed till date for its etiology. Derangement of lipid profile levels are known to cause endothelial dysfunction due to reduced bioavailability of nitric oxide and this endothelial dysfunction has been suggested to play a role in retinal exudates formation in diabetic retinopathy but this role of dyslipidemia in causation is also controversial.[4] Aim of the present study was to evaluate the level of serum lipid profile in patients with newly diagnosed type 2 diabetes mellitus with and without retinopathy.

Subjects and Methods

This present study was conducted in the Department of Ophthalmology, World College of Medical Sciences Research and Hospital, Jhajjar, in collaboration with the Department of Medicine and Biochemistry during the period from February 2019 to June 2019. Twenty eight (28) Patients with newly diagnosed type 2 diabetes mellitus without any signs of retinopathy were identified from outpatient services of department of Medicine and Twenty eight (28) Patients with newly diagnosed type 2 diabetes mellitus with retinopathy were identified from outpatient services of department of Ophthalmology along with 28 healthy subjects were taken as controls. Diabetic retinopathy was diagnosed after doing detailed dilated fundoscopic examination as per the standard criteria.[5]

Biochemical parameters studied:

After an overnight fasting 5 ml of venous blood sample was collected for the estimation of fasting plasma glucose, HbA1c and lipid profile were studied for following parameters.

1. Blood Glucose by GOD-POD methods.[6]
2. Glycated Haemoglobin (GHb) by cation exchange resin methods.[7]
3. Total Cholesterol (TC) by enzymatic end point CHODPOD methods.[8]
4. Triglyceride (TG) by enzymatic glycerol phosphate oxidase/peroxidase methods.[9]
5. High Density Lipoprotein-Cholesterol by direct enzymatic end point method.[10,11]
6. Low Density Lipoprotein -Cholesterol by Friedewald's formula.[12]
7. Very low Density Lipoprotein -Cholesterol by Friedewald'seqution.

LDL-c = Tc - HDL-c (TG/5)

Statistical analysis:

Statistical analysis of data was performed using the SPSS-16. For comparison of parameters between the diabetic with retinopathy, without retinopathy and normal healthy subjects, students t test was used. Statistical significance was considered at a 'p' value of <0.05 was considered significant.

Results & Discussion

Table 1: Shows the Mean & S.D. of with or without diabetic retinopathy

Variable	Diabetic retinopathy (N=28)	Without retinopathy (N=28)	P value
	Mean ± S.D.	Mean ± S.D.	
Age in Years	54.1±8.4	52.6±6.7	NS*
Fasting blood sugar (mg/dl)	152.3±44.9	146.9±54.7	0.76*
HbA1c (%)	9.8±2.75	9.04±1.98	0.54*
Triglycerides (mg/dl)	167.74±52.7	154.72±6.02	0.78*
Total cholesterol (mg/dl)	182.9±50.57	178.12±39.47	0.72*
Low density lipoproteins cholesterol (mg/dl)	113.5±36.05	106.3±32.4	0.42*
High density lipoproteins cholesterol (mg/dl)	38.5±12.2	38.77±11.34	0.12*
Very low density lipoproteins cholesterol (mg/dl)	35.2±10.4	29.0±9.21	0.09*

{Statistically significant (p<0.05), * Not Significant (p>0.05)}

A total of 84 subjects were included; 28 Patients with diabetic retinopathy and 28 Patients without diabetic retinopathy and 28 healthy subjects. The mean age of with or without diabetic retinopathy and healthy subjects was (54.1±8.4), (52.6±6.7) & 38.4 ±5.42 respectively [Table 1-

3]. Similarly, the Mean±S.D. of fasting blood sugar in patients with or without diabetic retinopathy and healthy subjects was {(152.3±44.9), (146.9±54.7) & (87.59+20.3)}; HbA1c was {(9.8±2.75), (9.04±1.98) & (5.9+0.62)}, triglycerides were {(167.74±52.7), (154.72±6.02) & (133.27±57.6)}; total cholesterol was {(182.9±50.57), (178.12±39.47) & (167.55±28.7)}; low density lipoprotein cholesterol was {(113.5±36.05), (106.3±32.4) & (96.8±16.5)}; high density lipoprotein cholesterol was {(38.5±12.2), (38.77±11.34) & (46.1±9.7)} and very low density lipoprotein cholesterol was {(35.2±10.4), (29.0±9.21) & (25.7±8.3)} respectively from table (1,2&3). A statistically significant difference was found in mean HbA1C and FBS in patients without retinopathy and healthy subjects (p<0.001; p<0.001) and in patients with diabetic retinopathy and healthy subjects (p<0.001; p<0.001). The difference was statistically not significant in between without retinopathy and with diabetic retinopathy (p=0.76; p=0.54) respectively. A statistically significant difference was found in mean high density lipoprotein-cholesterol value in patients without retinopathy and healthy subjects (p=0.03) and in diabetic retinopathy and healthy subjects (p=0.03). The difference was statistically not significant in between without retinopathy and with diabetic retinopathy (p=0.12). On comparing between with diabetic retinopathy and without retinopathy, it was observed that triglycerides levels were significantly increased (p>0.05), whereas low density lipoprotein and total cholesterol were not found to be significantly raised (p=0.42; p=0.72) respectively. This suggests that only increased triglycerides is associated with the increased incidence of diabetic retinopathy in diabetes mellitus subjects and not low density lipoprotein and total cholesterol.

Table 2: Shows the Mean & S.D. without diabetic retinopathy and healthy subjects

Variable	Without retinopathy (N=28)	Healthy Subjects (N=28)	P value
	Mean ± S.D.	Mean ± S.D.	
Age in Years	52.6±6.7	38.4 ±5.42	NS*
Fasting blood sugar (mg/dl)	146.9±54.7	87.59+20.3	<0.001
HbA1c (%)	9.04±1.98	5.9+0.62	<0.001
Triglycerides (mg/dl)	154.72±6.02	133.27±57.6	<0.04
Total cholesterol (mg/dl)	178.12±39.47	167.55±28.7	0.24*
Low density lipoproteins cholesterol (mg/dl)	106.3±32.4	96.8±16.5	0.20*
High density lipoproteins cholesterol (mg/dl)	38.77±11.34	46.1±9.7	0.03
Very low density lipoproteins cholesterol (mg/dl)	29.0±9.21	25.7±8.3	0.07*

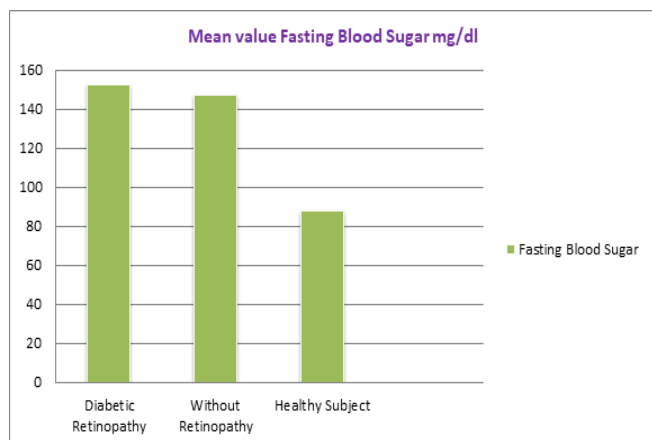{Statistically significant (p<0.05), * Not Significant (p>0.05)}

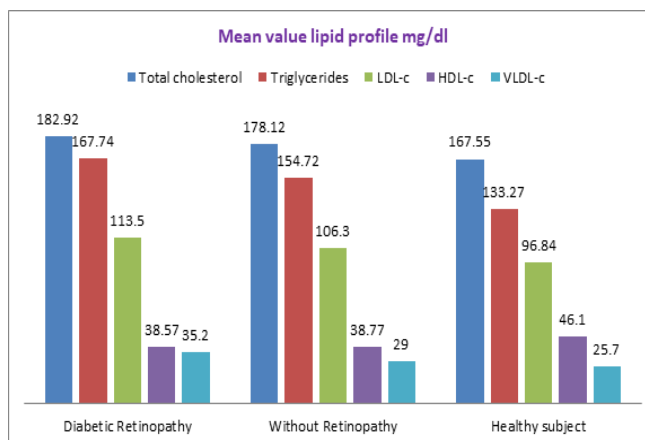**Figure 1: Shows the mean value of fasting blood sugar.**

Table 3: Shows the Mean & S.D. with diabetic retinopathy and healthy subjects

Variable	Diabetic retinopathy (N=28)	Healthy Subjects (N=28)	P value
	Mean ± S.D.	Mean ± S.D.	
Age in Years	54.1±8.4	38.4 ±5.42	NS*
Fasting blood sugar (mg/dl)	152.3±44.9	87.59+20.3	<0.001
HbA1c (%)	9.8±2.75	5.9+0.62	≤0.001
Triglycerides (mg/dl)	167.74±52.7	133.27±57.6	<0.04
Total cholesterol (mg/dl)	182.9±50.57	167.55±28.7	0.31*
Low density lipoproteins cholesterol (mg/dl)	113.5±36.05	96.8±16.5	0.23*
High density lipoproteins cholesterol (mg/dl)	38.5±12.2	46.1±9.7	0.03
Very low density lipoproteins cholesterol (mg/dl)	35.2±10.4	25.7±8.3	0.08*

{Statistically significant (p<0.05), * Not Significant (p>0.05)}

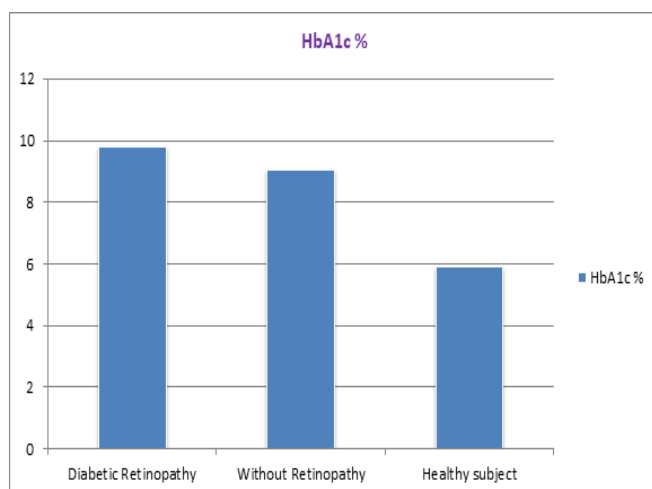**Figure 2: Shows the mean value of HbA1c.**

Figure 3: Shows the mean value of Lipid profile.

[Table 1-3 & Figure 3] compares the levels of total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol in patients with or without diabetic retinopathy and healthy subjects. On comparing between with diabetic retinopathy and healthy subjects, it was observed that triglycerides levels and high density lipoprotein cholesterol level were significantly increased (p<0.04,p=0.03)respectively where as low density lipoprotein cholesterol, very low density lipoprotein cholesterol and total cholesterol were not found to be significantly raised (p=0.20; p=0.07; p=0.24)respectively. This suggests that mainly increased triglycerides level and high density lipoprotein cholesterol levels are associated with the increased incidence of diabetic retinopathy in diabetes mellitus subjects as compared to healthy subjects and not low density lipoprotein cholesterol, very low density lipoprotein cholesterol and total cholesterol. On comparing between patients of diabetes mellitus without diabetic retinopathy and healthy subjects, it was observed that triglycerides levels and high density lipoprotein cholesterol level were significantly increased(p<0.04,p=0.03)respectively where as low density lipoprotein cholesterol, very low density lipoprotein cholesterol and total cholesterol were not found to be significantly raised (p=0.23; p=0.08; p=0.31)respectively. This suggests that mainly raised triglycerides level and high density lipoprotein cholesterol are associated in diabetes mellitus subjects as compared to healthy subjects and not low density lipoprotein cholesterol, very low density lipoprotein cholesterol and total cholesterol. Various studies have proven the role of elevated serum lipids with macro vascular complications of diabetes mellitus like coronary artery disease but, studies of association of lipids with specific micro vascular complications of diabetes mellitus like retinopathy have shown varying results. Dornan et al (1982),[13] first showed the an association of low density lipoprotein cholesterol with diabetic retinopathy. In Wisconsin epidemiological study of diabetic retinopathy, Klein et al (1999),[14] correlated raised cholesterol levels with macular hard exudates. Early treatment diabetic retinopathy study associated low density lipoprotein and

total cholesterol with the onset and severity of retinal hard exudates (Chew et al, 1966).[15] Severity of retinopathy was positively associated with triglycerides in type I diabetes mellitus and negatively associated with high density lipoprotein choestrol in DCCT/ EDIC cohort (Lysons et al, 2004).[16] In Wisconsin epidemiological study of diabetic retinopathy, Klein et al (1999),[14] correlated raised cholesterol levels with macular hard exudates. Early treatment diabetic retinopathy study associated low density lipoprotein and total cholesterol with the onset and severity of retinal hard exudates (Chew et al, 1966).[15] Severity of retinopathy was positively associated with triglycerides in type I diabetes mellitus and negatively associated with high density lipoprotein cholesterol in DCCT/ EDIC cohort (Lysons et al, 2004).[16] Mohan et al (1984),[17] reported an association between raised low density lipoprotein and macular edema in the Indian population. Later Rema et al (2006),[18] showed association of triglycerides with diabetic retinopathy and low density lipoprotein with diabetic macular edema in Chennai Urban Rural Epidemiology Study Eye study (CURES). However, Larsson et al (1999),[19] and Hove et al (2004),[20] found no association between triglycerides, total cholesterol and high density lipoprotein cholesterol with diabetic retinopathy. Kulshreshtha et al (1979),[21] observed raised levels of cholesterol and NEFA (non esterified fatty acids) in patients of diabetic retinopathy. Benarous et al (2011),[22] reported that phospholipids are not associated with diabetic retinopathy but with Clinically significant macular oedema. Sasongko et al (2011),[23] showed that apolipoprotein AI is inversely related and apolipoprotein B is directly related to diabetic retinopathy and are strong biomarkers of diabetic retinopathy than lipid profile in Australian population. Ozer et al (2009),[24] found no correlation between serum lipids and macular edema in diabetic patients. Sachdev&Sahni (2010),[25] proved that total cholesterol and low density lipoprotein are risk factors for retinal hard exudates in Type 2 diabetes mellitus in North Indian population. Keech et al (2007),[26] showed that lipid lowering agent like finofibrate, decreases the progression of diabetic retinopathy. Uçgun et al (2007),[27] showed that total cholesterol and low density lipoprotein are elevated in patients with macular edema and hard exudates. In the present study, it was found that total cholesterol, triglycerides and low density lipoprotein cholesterol levels were higher in patients with or without diabetic retinopathy as compared to the healthy subjects. This is due to the increased flow of glucose and fatty acids to liver due to lack of insulin. Decreased clearance of triglycerides and low density lipoprotein cholesterol is due to over production of apolipoprotein B and low lipoprotein lipase activity. On comparing between diabetic retinopathy and healthy subjects it was observed that triglycerides levels were significantly increased ($p<0.04$), whereas low density lipoprotein and total cholesterol were not found to be significantly raised ($p=0.20$; $p=0.24$) respectively. The raised triglyceride levels leads to increased blood viscosity and altered fibrinolytic activity which leads to formation of hard exudates. Also, triglycerides incorporate into the cell membrane, altering its fluidity and permeability which leads

to hemorrhage and oedema. This also leads to endothelial cell dysfunction and local inflammatory response releasing cytokines and growth factors which are responsible for neovascularisation in retina (Joussen et al, 2001).[28] In the present study ,total cholesterol and low density lipoprotein levels were not found to be significantly raised in those with diabetic retinopathy as compared to those without diabetic retinopathy. This study suggests the role of increased triglycerides and high density lipoprotein cholesterol and not total cholesterol and low density lipoprotein in the incidence of retinopathy in Type 2 diabetes mellitus. The mechanisms by which high serum lipids cause the development and progression of diabetic retinopathy are not fully understood. It has been postulated that an increase in blood viscosity and alterations in the fibrinolytic system occur in hyperlipidaemia and lead to the formation of hard exudates.[29] Also, incorporation of triglycerides into the cell membrane may lead to changes in membrane fluidity and leakage of plasma constituents in the retina.[30] This results in haemorrhage and oedema in the retina. Also, high lipid levels are known to cause endothelial dysfunction through a local inflammatory response,[31,32] with subsequent release of cytokines and growth factors, hypoxia, etc. In animal models it has been shown that endothelial dysfunction in the diabetic vasculature results in blood–retinal barrier breakdown.[32-34]

Conclusion

Various studies have proven the role of elevated serum lipids with macro vascular complications of diabetes mellitus like coronary artery disease but, studies of association of lipids with specific micro vascular complications of diabetes mellitus like retinopathy have shown varying results. Our study suggests that both triglycerides and high density lipoprotein cholesterol are increased in newly diagnosed type 2 diabetes mellitus irrespective of with or without diabetic retinopathy.

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
2. Prevention of Blindness from Diabetic Retinopathy. Report of a WHO Consultation, Geneva; November, 2005.
3. Yau JW, Rogers SL, Kawasaki R, Lamoureux EL, Kowalski JW, Bek T, et al. Meta-Analysis for Eye Disease (META-EYE) Study Group. Global prevalence and major risk factors of diabetic retinopathy. Diabetes Care 2012;35:556-64.
4. Landmesser U, Hornig B, Drexler H. Endothelial dysfunction in hypercholesterolemia: mechanisms, pathophysiological importance, and therapeutic interventions. SeminThrombHemost. 2000;26(5):529-37.
5. Wu L, Fernandez-Loaiza P, Sauma J, Hernandez-Bogantes E, Masis M. Classification of diabetic retinopathy and diabetic macular edema. World J Diabetes. 2013;4(6):290-4.
6. Bergmayer H.V. – "Methods of Enzymatic Analysis",A.P., N.Y. 1974, Page 1196.
7. Gabby, K H et al. – J. Clin. End. Met. 44:859,1977.
8. Richmond W. – Preparation and properties of cholesterol oxidase from Nocardia sp. and its application to the enzymatic assay of total

cholesterol in serum. Clin Chem. 19: 1350-1356, 1973.

9. Foosati P. and Prencipe L. – Serum triglyceride determined colorimetrically with an enzyme that produce hydrogen peroxide. Clin Chem. 28: 2077-2080, 1982.

10. Rifai N. and Warnick G.R., Ed.- Laboratory measurements of lipids, lipoproteins and apolipoproteins. AACC press, Washington, DC, USA 1994.

11. Burtis, C.A. and Ashwood, E.R. Ed. – Tietz Textbook of clinical chemistry, 2nd Ed, Saunders, Philadelphia, 1994.

12. Friedewald W.T., Levy R.I., Fredrickson D.S., clin Chem. 18:499, 1972.

13. Dornan TL, Carter RD, Bron AJ, Turner RC, Mann JI: Low density Lipoprotein cholesterol: An association with severity of diabetic retinopathy. Diabetologia, 1982;22(3):167-170.

14. Klein BE, Klein R ,Moss SE: Is serum cholesterol associated with progression of diabetic retinopathy or macular oedema in persons with younger-onset diabetes of long duration? American Journal of ophthalmology, 1999;128(5):652-654.

15. Chew EY, Klein ML, Ferris FL (3rd), Remaley NA, Murphy RP, Chantry K, Hoogwerf BJ, Miller D: Association of elevated serum lipid levels with retinal hard exudate in diabetic retinopathy. Early treatment diabetic retinopathy study (ETDRS) Report 22. Archives of Ophthalmology, 1996;114(9):1079-1084.

16. Lyons TJ, Jenkins AJ, Zheng D, Lackland DT, McGee D, Garvey WT, Klein RL: Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. Investigative Ophthalmology Visual Science, 2004;45(3):910-918.

17. Mohan R, Mohan V, Susheela L, Ramachandrann A, Viswanathan M: Increased LDL cholesterol in Non- Insulin-dependent diabetics with maculopathy. ActaDiabetologica Latina, 1984;21(1):85-89.

18. Rema M, Srivastava BK, Anitha B, Deepa R, Mohan V: Association of serum lipids with diabetic retinopathy in urban South Indians-the Chennai Urban Rural Epidemiology Study (CURES) Eye Study-2. Diabetic Medicine, 2006;23(9):1029-1036.

19. Larsson LI, Alm A, Lithner F, Dahlen G, Bergstrom R: The association of hyperlipedemia with retinopathy in diabetic patients aged 15-50years in the county of Umea. ActaOphthalmologicaScandinavica, 1999;77(5):585- 591.

20. Hove MN, Kristensen JK, Lauritzen T, Bek T: The prevalence of retinopathy in an unselected population of type 2 diabetes patients from Arhus County, Denmark. ActaophthalmologicaScandinavica 2004; 82(4):443- 448.

21. Kulshreshtha OP, Nayar SK, Sharma DP: Role of serum lipids in diabetic retinopathy. Indian Journal of Ophthalmology, 1979; 27(4);116-118.

22. Benarous R, Sasongko MB, Qureshi S, Fenwick E, Dirani M, Wong TY et al. Differential association of serum lipids with diabetic retinopathy and diabetic macular edema. Invest Ophthalmol Vis Sci. 2011;52(10):7464-9.

23. Sasongko MB, Wong TY, Nguyen TT, Kawasaki R, Jenkins A, Shaw J, Wang JJ: Serum Apolipoprotein AI and B are strong biomarkers of diabetic retinopathy than traditional lipids. Diabetes Care, 2011;34(2):474-479.

24. Ozer PA, Unlu N, Demir MN, Hazirolan DO, Acar MA, Duman S: Serum lipid profile in diabetic macular edema. Journal of Diabetes and its Complications, 2009;23(4): 244-248.

25. Sachdev N, Sahni A: Association of systemic risk factors with the severity of retinal hard exudates in a north Indian population with type 2 diabetes. Journal of Postgraduate Medicine, 2010;56(1):3-6.

26. Keech AC, Mitchell P, Summanen PA, O'Day J, Davis TM, Moffitt MS, Taskinen MR, Simes RJ, Tse D, Williamson E, Merrifield A, Laatikainen LT, d'Emden MC, Crimet DC, O'Connell RL, Colman PG: Effect of finofibrate on the need for laser treatment for diabetic retinopathy (FIELD study): a randomised controlled trial. Lancet, 2007;370(9600):1687-1697.

27. Uçgun NI, Yildirim Z, Kiliç N, Gürsel E: The importance of serum lipids in exudative diabetic macular edema in type 2 diabetic patients. Annals of the New York Academy of Sciences, Biogerontology: Mechanism and interventions, 2007;1100:213-217.

28. Joussen AM, Murata T, Tsujikawa A, Kirchhof B, Bursell SE, Adamis AP: Leucocyte-mediated endothelial cell injury and death in Diabetic retina. American Journal of Pathology, 2001;158(1):147-152.

29. Freyberger H, Schifferdecker E, Schatz H. Regression of hard exudates in diabetic background retinopathy in therapy with etofibrateantilipemic agent. Med Klin 1994; 89: 594–597.

30. Ebeling P, Koivisto VA. Occurrence and interrelationships of complications in insulin-dependent diabetes in Finland. ActaDiabetol 1997; 34: 33–38.

31. Langeler EG, Snelting-Havinga I, Van Hinsbergh VWM. Passage of low density lipoproteins through monolayers of human arterial endothelial cell: effects of vasoactive substances in an in vitro model. Arteriosclerosis 1989; 9: 550–559.

32. Rangaswamy S, Penn MS, Saidel GM, Chisolm GM. Exogenous oxidized low-density lipoprotein injures and alters the barrier function of endothelium in rats in vivo. Circ Res 1997; 80: 37–44.

33. Joussen AM, Murata T, Tsujikawa A, Kirchhof B, Bursell SE, Adamis AP. Leukocyte-mediated endothelial cell injury and death in the diabetic retina. Am J Pathol 2001; 158: 147–152.

34. Joussen AM, Poulaki V, Mitsiades N, Kirchhof B, Koizumi K, Dohmen S et al. Nonsteroidal anti-inflammatory drugs prevent early diabetic retinopathy via TNF-alpha suppression. FASEB J 2002; 16: 438–440.