

Blood Viscosity, Lipid Profile, and Lipid Peroxidation in Type-1 Diabetic Patients with Good and Poor Glycemic Control

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Abstract

Background: In diabetic patients, persistent hyperglycemia and poor glycemic control cause disturbances of lipid profiles, especially an increased production of oxygen free radicals. Lipid peroxidation has been considered to be a pathogenic factor of diabetic complications in Type-1 Diabetes mellitus. **Aims:** The aim of the present study was to investigate the effect of glycemic control on blood viscosity, lipid profile, and lipid peroxidation in Type-1 Diabetic subjects. **Materials and Methods:** The study included three groups; Group-I (age-matched healthy control subjects, $n = 50$), Group-II (Type-1 Diabetics with good glycemic control, $n = 10$), and Group-III (Type-1 Diabetics with poor glycemic control, $n = 15$). The Type 1 diabetic patients with duration of diabetes for more than 5 years were taken. Blood samples of all subjects were analyzed for all biochemical, hematological, and oxidative stress parameters. **Results:** The Erythrocyte malondialdehyde level was non-significantly changed ($P = NS$) in group-II patients but significantly increased ($P > 0.001$) in group-III patients, and no significant changes were found ($P = NS$) in Blood viscosity of both the groups (group-II and group-III), as compared to healthy control subjects (group-I). **Conclusions:** Our findings suggest that the monitoring of Oxidative stress and Blood Viscosity in poorly controlled Type-1 diabetic patients may be very useful marker of diabetic complications.

Keywords: Blood viscosity, Glycemic control, Lipid peroxidation, Lipid profile, Type-1 diabetes mellitus

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Introduction

Diabetes mellitus is a common metabolic disorder resulting from defects in insulin secretion or action or both, which is characterized by hyperglycemia often accompanied by glycosuria, polydipsia, and polyuria^[1] resulting from an absolute or relative deficiency of insulin secretion or action.^[2] Insulin is the key hormone in blood glucose homeostasis.^[3] Dyslipidaemia is a common feature of diabetes even with good glycemic control.^[4] The key features of this diabetic dyslipidaemia are elevated level

of triglyceride and LDL-c.^[5] Improved metabolic control may decrease very-low-density lipoprotein cholesterol (VLDL-Cholesterol) and low-density lipoprotein cholesterol (LDL-Cholesterol).^[6] The hyperglycemia or dyslipidaemia easily induce serious oxidative stress that causes serious cellular dysfunction as well as hematic and vascular complications in diabetic patients.^[7,8] Persistent hyperglycemia causes increased production of free radicals, especially reactive oxygen species (ROS), for all tissues from glucose auto-oxidation and protein glycosylation.^[9] Noberasco *et al.*^[10] and Gallon *et al.*^[11] reported that MDA is the most commonly used marker of lipid peroxidation in diabetes mellitus. Jenkins *et al.* (1996) reported a normal susceptibility of LDL to *in vitro* oxidation in well-controlled Insulin dependent Diabetes Mellitus (IDDM) patients.^[12]

Blood and plasma viscosity have emerged as independent risk factors for atherothrombotic vascular disease.^[13] Fibrinogen is a risk factor for

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DOI:
10.4103/1947-2714.118925

atherothrombotic disease.^[14] Fibrinogen is an important determinant of blood viscosity that results from Fibrinogen-induced erythrocyte aggregation and its contribution to plasma viscosity.^[15] Defeo *et al.*^[16] reported that fibrinogen synthesis is inhibited by the administration of insulin, hyper fibrinogenemia associated with insulinopemia.

Materials and Methods

The present study included 25 Type-1 diabetic patients (without complication) and 50 age-matched normal healthy controls who volunteered. The patients were recruited from the wards and OPDs of the Department of Medicine, Surgery, G.R. medical College and J.A. Hospital, Gwalior, M.P., India. The study was approved by the institutional ethical committee and written consent was also taken from the patients. These subjects were divided into three groups:

Group I: This group consisted of age-matched healthy control ($n = 50$).

Group II: This group consisted of good glyceemic control ($n = 10$) Type-1 subjects.

Group III: This group consisted of poor glyceemic control ($n = 15$) Type-1 subjects.

Controls were defined as not having a major medical illness, no hospital admissions, no current medication, and a subjective perception of good health. None of the healthy subjects received any medication and trace element supplement in the previous 2-3 months.

All diabetics were on insulin therapy since the last 5 to 15 years. The blood sample was taken from diabetic patients and healthy controls after an overnight fast under all aseptic precautions for analysis. All the samples were analyzed on the same day of collection. This sample was distributed in the following vials:

Ethylene di-amine tetra acetic acid (EDTA) sample for estimation of Fasting plasma glucose (FPG), plasma fibrinogen, Hematocrit, and Erythrocyte malondialdehyde (E-MDA). Citrated sample for estimation of Glycosylated hemoglobin (HbA_{1c}), Plain vial (Serum) for estimation of Total cholesterol (TC), Triglycerides (TG), VLDL-c, and HDL-c.

FPG was estimated by the method of Glucose Oxidase-Peroxidase (GOD-POD) by Trinder,^[17] Glycosylated hemoglobin was measured by spectrophotometric method,^[18] Triglyceride estimated by Von handle method,^[19] Total cholesterol done by Ferric chloride method,^[20] HDL cholesterol by Bernstein method,^[21] LDL and VLDL cholesterol were calculated by Friedwal's formula.^[22]

Friedwal's formula for LDL Cholesterol: Total cholesterol minus high-density lipoprotein (HDL) cholesterol minus VLDL cholesterol (estimated as triglyceride multiplied by 0.46).

LDL cholesterol (mmol/L) = Total cholesterol – HDL Cholesterol – VLDL cholesterol

Friedwal's formula for VLDL Cholesterol:

VLDL cholesterol (mmol/L) = $0.46 \times$ Triglyceride

E-MDA for lipid peroxidation was done by Bidder and Jaeger Method,^[23] Plasma fibrinogen was determined by Tyrosine (Lempert) method,^[24] Hematocrit was determined by Wintrobe's method,^[25] and Blood viscosity was calculated by Merrill's formula.^[26]

The blood viscosity was calculated by the formula as described by Merrill.

Blood viscosity (YSS) = $13.5 \times 10^{-6} \times$ (Fibrinogen Concentration in gm%)² \times (Hct-6)³

Where: YSS = Yield shear stress, gm = Gram, Hct = Haematocrit

Statistical study

All results were expressed in Mean \pm SD. The statistical analysis was performed using Statistical Product and Service Solutions (SPSS) version 7.0. Pearson correlation coefficients were calculated to analyze relationships between biochemical parameters. The level of significance was considered as P value < 0.05 .

Results

Table 1 shows the levels of fasting blood sugar, glycosylated hemoglobin, lipid profile, and E-MDA in Type-1 diabetic patients and healthy control subjects. There were significant increases in Group-III ($n = 15$) patients in the levels of FPG and ($P < 0.001$) and TC ($P < 0.001$), TG ($P < 0.05$), and HDL ($P < 0.001$), while Group-II ($n = 10$) patients had non-significant changes in total cholesterol, VLDL-c, and LDL-c when compared with normal healthy subjects.

Table 2 shows the levels of plasma fibrinogen, hematocrit, and blood viscosity in Group-I, Group-II, and Group-III. Plasma fibrinogen, hematocrit, and blood viscosity ($P = \text{NS}$) were non-significantly changed as compared with those of healthy control subjects [Table 2]. These results indicate that in Group-II ($n = 10$), good glyceemic control helps in lowering the level of lipid profile and blood viscosity, which may contribute to the prevention of onset of diabetic complications.

Table 3 shows the correlation between blood viscosity *vs* fasting blood sugar, glycosylated hemoglobin, lipid profile, plasma fibrinogen, hematocrit, and E-MDA. There was significant correlation found between blood viscosity *vs* TC ($P < 0.001$), LDL-C ($P < 0.001$), plasma fibrinogen ($P < 0.05$), and hematocrit ($P < 0.001$), whereas non-significant correlation have been found between blood viscosity *vs* FBS, TG, HDL-c, VLDL-c, and E- MDA ($P = NS$).

Discussion

Blood viscosity is a basic biological parameter that affects blood flow both at large arteries and in the microcirculation. There is a sufficient evidence that

the elevated blood viscosity is a pathogenic factor of diabetic microangiopathy, altering microcirculation and leading to insufficient tissue nutrition.^[27] A number of researchers found that the blood viscosity was altered in diabetes.^[28-30] As the osmolarity of the blood increases due to increased sugar level, the capillary permeability increases, thus increasing hematocrit and subsequently the blood viscosity.^[31] Similar findings have been observed by Lowe *et al.*^[32] and Langer *et al.*^[33] that osmotic diuresis is caused by hyperglycemia, leading to decreased plasma volume and increased hematocrit. Barnes *et al.*^[34] reported that both hematocrit and blood viscosity decreased after institution of good diabetic control. Diabetes patients had a higher blood viscosity than healthy people.^[35,36]

Table 1: FBG, HbA_{1c}, Lipid profile, and E-MDA in Group-I, Group-II, and Group-III subjects

Variables	Group-I (n=50)	Group-II (n=10)	Group-III (n=15)	P value
Age (Yrs.)	42.02 ±11.46	26.2±4.73	33.7±10.71	<0.05 ^[1] , <0.02 ^[2]
Duration of Diabetes (Yrs.)	—	6±1.05	10.8 ±7.95	—
Fasting Plasma Glucose (mmol/L)	4.27±0.42	4.91±0.39	9.84±2.20	<0.001 ^[1,2]
HbA _{1c} (MH/M Hb)	0.26±0.009	0.45±0.053	0.62±0.29	<0.001 ^[1,2]
Triglyceride (mmol/l)	1.44 ±0.28	1.23±0.27	1.94±0.57	<0.05 ^[1] , <0.01 ^[2]
Total cholesterol (mmol/l)	4.79±0.57	4.88±0.45	5.94±1.03	NS ^[1] , <0.001 ^[2]
HDL-c (mmol/l)	1.01±0.17	1.21±0.17	1.16±0.22	<0.001 ^[1] , <0.02 ^[2]
VLDL-c (mmol/l)	0.65±0.13	0.56 ±0.12	0.92±0.23	NS ^[1] , <0.001 ^[2]
LDL-c (mmol/l)	3.13±0.60	3.11±0.42	3.85±0.98	NS ^[1] , <0.01 ^[2]
E-MDA (n mol/Hb/2 hrs.)	125.11±12.48	126.41±17.72	162.48±30.52	NS ^[1] , <0.001 ^[2]

The values are mean± Standard deviation (SD); P values: ^[1]p, Control vs. Group-II Diabetic Patients; ^[2]p, Control *vs* Group-III Diabetic patients

Table 2: Levels of plasma fibrinogen, hematocrit, and blood viscosity in Group-I, Group-II, and Group-III subjects (Mean±SD)

Variables	Group-I (n=50)	Group-II (n=10)	Group-III (n=15)	P value
Plasma Fibrinogen (mg%)	255.42±9.57	253.9±20.93	273.93±59.70	P=NS
HCT %	41.32±3.43	41.4±3.30	43.13±6.92	
B.Vis. (YSS/dyne cm ²)	0.036±0.020	0.039±0.012	0.056±0.040	

P=NS (Non-significant at all levels), healthy control (Group I) *vs.* Type 1 diabetic subjects (Group II, Group III); Hct=Haematocrit

Table 3: Correlation of blood viscosity with FPG, HbA_{1c}, lipid profile, plasma fibrinogen, E-MDA, and hematocrit in Group-I, Group-II, and Group-III subjects

Variables	Group-I (n=50)	Group-II (n=10)	Group-III (n= 15)
FBS	r=-0.219, P=NS	r=0.138, P=NS	r=0.094, P=NS
HbA1c	r=0.265, P=NS	r=-0.105, P=NS	r=-0.245, P=NS
TC	r=-0.097, P=NS	r=-0.513, P<0.05	r=0.842, P<0.001
TG	r=-0.168, P=NS	r=-0.361, P=NS	r=0.159, P=NS
HDL-c	r=-0.093, P=NS	r=-0.306, P=NS	r=0.242, P=NS
LDL-c	r=-0.062, P=NS	r=-0.320, P=NS	r=0.812, P<0.001
VLDL-c	r=-0.0159, P=NS	r=-0.354, P=NS	r=0.101, P=NS
P.Fibrinogen	r=0.403, P<0.005	r=0.543, P<0.05	r=0.586, P<0.01
E-MDA	r=0.196, P=NS	r=-0.428, P=NS	r=-0.060, P=NS
Hct.	r=-0.867, P<0.0005	r=0.835, P<0.005	r=0.611, P<0.005

Pearson correlation coefficient (r) is significant at the $P < 0.05$, $P < 0.01$, $P < 0.001$, $P < 0.005$, $P < 0.0005$ level (1 tailed), Pearson correlation coefficient (r) is non-significant; P: NS; HDL-c: High-density lipoprotein Cholesterol LDL-c: Low-density lipoprotein-Cholesterol; VLDL-c: Very-low-density lipoprotein-Cholesterol; E-MDA: Erythrocyte malondialdehyde

There are convincing experimental and clinical evidences that the generation of ROS is increasing in both types of diabetes and that the onset of diabetes is closely associated with oxidative stress.^[37,38] Free radicals are formed disproportionately in diabetes by glucose autoxidation, polyol pathway, and non-enzymatic glycation of proteins.^[39] The increased production and/or ineffective scavenging of such free radicals may play a crucial role in determining tissue injury, and increased lipid peroxidation which leads to complications of diabetes mellitus.^[40] The increased level of glycosylated hemoglobin was observed in the diabetic patients and this increase is directly proportional to the blood glucose level.^[41] This suggests the increase in oxidative stress due to hyperglycemia. Increased lipid peroxidation impairs membrane function by decreasing membrane fluidity and changing the activity of membrane-bound enzymes and receptors. The products of lipid peroxidation are harmful to most cells in the body and are associated with a variety of diseases, such as atherosclerosis and brain damage.^[42]

In our study, a significant increase of MDA was observed in the Group-III diabetic patients. We have found that after taking insulin, some diabetic subjects had poor glycemic control and some had good glycemic control. A study was carried out in these two groups and observed that glycemic control plays a very important role and could help in reducing the onset of diabetic complications. An increase in plasma TG concentration signifies and elevation of the VLDL and /or chylomicron concentrations as suggested previously.^[43] Patients with IDDM who are in extremely poor metabolic control develop severe hypertriglyceridemia, primarily due to accumulation of chylomicrons.

Patients have taken insulin therapy, and fibrinogen synthesis is inhibited by the administration of insulin.^[44,45] Lower fibrinogen and hematocrit level can help to affect the blood viscosity level.

A further research is needed on large sample size to enhance our present understanding statistically, especially on Group-I and Group-II of Type-1 Diabetic patients.

Conclusions

The increased efficiency of oxidative stress in Type-1 diabetic patients with poor glycemic control constitute the pathogenic link between hyperglycemia and development of endothelial dysfunction. Moreover, blood viscosity was not increased due to insulin administration in both the groups of Type-1 Diabetes mellitus patients.

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How to cite this article: Mishra N, Singh N. Blood viscosity, lipid profile, and lipid peroxidation in type-1 diabetic patients with good and poor glycemic control. *North Am J Med Sci* 2013;5:562-6.

Source of Support: Nil. **Conflict of Interest:** None declared.