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Coagulation Factors Evaluation in NIDDM Patients

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ABSTRACT

Diabetes mellitus is the major risk factor for vascular diseases presumably by inducing hypercoagulable state leading to thrombotic plaque in small blood vessels. There are miscellaneous reports showing the intervention of some coagulation factors/co-factors, especially I, II, V, VII, VIII, IX, X, XI, vonWillebrand and tissue factor in micro and macrovascular complications. In the present we used 40 uniformed NIDDM patients and 21 healthy volunteers as control for the study. Based on the results obtained for coagulation tests such as PT, APTT and the specific activity or concentration determination for factors I, II, V, VII, VIII, IX, X, X and von Willebrand, we made a new comprehensive comparison between NIDDM and healthy groups. Present results show the major interference of intrinsic and common pathway of coagulation cascade factors and less of extrinsic pathway in hypercoagulability state in NIDDM patients. Present results show that there are significant increase ($p<0.05$) in plasma activities of coagulation factors, FII, FIX, FXI in intrinsic and common pathways and significant decrease in activity of factor VIII and prolonged prothrombin time in NIDDM patients ($n = 40$) in contrast to normal controls ($n = 21$). This finding leads us to conclude that this pattern could be used as diagnostic criteria for the presence of hypercoagulable state and as prognostic tools for susceptibility to vascular disease in NIDDM patients. Second, our findings in conjunction with patients history indicates that the presence of hypercoagulable state is not sufficient to induced vascular problems in NIDDM and instead it need additional triggering factors to initiate vascular thrombotic problems e.g., endothelium damage.

Key words: Hypercoagulable, coagulation factors, common pathway, intrinsic pathway, NIDDM

INTRODUCTION

Diabetes mellitus (insulin dependent, IDDM and or non-insulin dependent, NIDDM) is the major risk factor for micro and macrovascular complications leading to hypertension, peripheral arterial disease, endothelial dysfunction, congestive heart failure and finally heart attack (Ibbotson *et al*., 1992; Tripodi *et al*., 2010; Reverter *et al*., 1997). One of the mostly studied mechanisms for diabetes mellitus pathogenesis is the abnormality exerted in coagulation haemostasis and platelets dysfunction. However, it had been reported that reduced activity of fibrinolytic system accelerates atherogenesis in diabetic patient (Carr, 2001; Collier *et al*., 1992;

Carmassi *et al.*, 1992). There are miscellaneous and somehow conflicting reports regarding the changing pattern in plasma levels for coagulation factors e.g. I, VII, VIII, IX, XI, vonWillebrand factor (vWF) and Tissue Factor (TF) (El-Hagracy *et al.*, 2010; Cardigan *et al.*, 1999; Lohmann *et al.*, 2009; Laakso and Lehto, 1998; Vaidyula *et al.*, 2009; Falati *et al.*, 2002; Mackman, 2004). Some studies convey significant increase in plasma concentrations of coagulation factors for NIDDM patients in contrast to normal controls; some others show that the increments are not noticeable (Kluft and Jespersen, 2002; Wakabayashi and Masuda, 2002; Bolaman *et al.*, 2007; Schneider *et al.*, 2005; Madan *et al.*, 2010). Factor I (fibrinogen), the precursor of fibrin, play critical role in blood viscosity. Increased concentration of fibrinogen (hyperfibrinogenemia) in uncontrolled NIDDM patients is suspicious to takes part in vascular damage induction (Zachary and Bloomgarden, 2011; Makin *et al.*, 2004). Instead, in well controlled NIDDM patients there is no report showing hyperfibrinogenemia (El-Khawand *et al.*, 1993; Dubrey *et al.*, 1994; Borissoff *et al.*, 2010). Accordingly, there are many studies support that induction and progression of vascular damage could leads to inflammation and increased TF production and is concomitant with hyperfibrinogenemia (Lowe *et al.*, 1980; Bots *et al.*, 2002; Ray *et al.*, 1994; Weiss *et al.*, 2000; Lusis, 2000; Lee *et al.*,1996; Harris *et al.*, 1978; Wiseman *et al.*, 1989; Merlo *et al.*, 2002). Diabetic hyperglycemia is the prominent causal candidate for induction of endothelium damage by accelerating glycosylation or sorbitol pathways, therefore long term hyperglycemia in NIDDM patients could be used as proof for the prediction of accelerated endothelial damage (Li *et al.*, 2003). Increased levels of TF in NIDDM patients activates factor VII and changed it to VIIa. Activated factor VII (VIIa), triggers the extrinsic pathway of coagulation cascade via converting factor X to Xa. Contrariwise, high levels of TF could be used as criteria for the presence of endothelium damage (Wiseman *et al.*, 1989; Merlo *et al.*, 2002). Diabetic hypertriglyceridemia, the additional risk factor for cardiovascular problems, in addition to increased levels of TF and VII gives a bad prognosis for serious fatal ischemic heart disease. Activated factor VIII (VIIIa) is another threatening factor for cardiovascular disease. Factor VIIIa, in the intrinsic pathway of coagulation cascade catalyzes the activation of factor IX to IXa. Factor VIII circulates in the plasma as complex with vonWillebrand factor (vWF) to protect FVIII from proteolysis and prolonged its half life. Abnormally higher levels of VIII were reported in patients with cardiovascular disease (Voetsch and Loscalzo, 2004; Karami *et al.*, 2010; Koster *et al.*, 1995; Hajifathali *et al.*, 2005; Whiteley *et al.*, 2009; Kucharska-Newton *et al.*, 2009; Meade *et al.*, 1986; Meade *et al.*, 1994). In NIDDM increased levels of VIII were seen with uncertain statistical correlations to vascular complications (Berliner *et al.*, 2002; Eteng *et al.*, 2008; Behnam-Rassouli *et al.*, 2010). There are some reports showing insignificant difference in VIII levels between NIDDM and control groups. However, increased level of VIII is more evident in patients with higher vWF concentration. A like TF and fibrinogen, vWF is also known as a marker for endothelium damage and in turn is increased in NIDDM (Frankel *et al.*, 2008; Elhadd *et al.*, 2001; Lip and Blann, 1997; Bonetti *et al.*, 2003; Meigs *et al.*, 2006; Woodburn *et al.*, 1995; Kessler *et al.*, 1998; Thor *et al.*, 2002). There are few reports demonstrates the involvement of clotting factor IX and XI in vascular complications but their increase in NIDDM are reported to not be striking (Minnema *et al.*, 2000; Barillari *et al.*, 2009; Rezaeian *et al.*, 2006). According to pervious studies and in a new effort, we measured the specific activity/concentration of coagulation factors in 40 NIDDM patients in early stage of NIDDM onset and without any vascular complication in comparative study with 21 normal controls. The major aim of the present study was to survey the changes in coagulation factors concentration in early stage of NIDDM and to evaluate the extent of intrinsic, extrinsic and common pathways of coagulation cascade involvement in hypercoagulable state in NIDDM patients.

MATERIALS AND METHODS

Subjects selection: In the present study, 40 NIDDM patients with no cardiovascular history where chosen from 2054 diabetic patients for this study. NIDDM patients were selected based on their medical history obtained from Dezfoul Ganjavian Hospital, Khuzestan Province, Iran. All NIDDM patients were diagnosed from 2007 to 2010 with the ages ranging from 45 to 65 years. All participants gave written informed consent and all subjects were informed about the study and signed informed this forms. NIDDM patients and volunteers were excluded if they had history or even manifestation of cardiovascular disease, peripheral vascular disease, coagulation disorders, neuropathy, nephropathy, insulin therapy, psychiatric illness, smoking and any acute or chronic disease. The only medication used by diabetics was anti-diabetic metformin pill having no insulin. Tewnty one healthy volunteers with the same age range were chosen as control group.

Sample preparation: Blood samples were drawn in the morning and after at least 12 h of fasting from NIDDM and control groups. The biochemical and hematological parameters were analyzed in Ganjavian hospital laboratory (Dezfoul, Khuzestan Province, Iran). In order to measure coagulation factors the blood samples were delivered to Ahwaz Shafa Hospital laboratory (Ahwaz, Iran) in the same day. The 2 mL of whole blood, 2×9 mL were collected into two plastic syringes (Monovette®, Sarstedt, Numbrecht, Germany), each containing 0.2 mL 0.106 M tri sodium citrate. Plasma was prepared by centrifuging twice at 1500x g for 10 min each at 15-18°C and was then stored in polypropylene tubes at -70°C until measurement. All samples were assayed in duplicated method. Any plasma sample with evidence of hemolysis was discarded.

Analytical methods: Weight and height of participants were determined in light clothing and without shoes. Portable calibrated electronic weighing scale and portable measuring inflexible bars were used. Body Mass Index (BMI) was calculated as weight (in kilograms) divided by height (in meters) square. Blood pressure was measured with a calibrated Omron M7 sphygmomanometer (HEM-780-E). The mean value of three measurements, made at intervals of 10 min, was used for analysis. Fasting plasma glucose of subjects measured by the enzymatic colorimetric (GOD/PAP) method (ParsAzmun Co. Kits, Karaj, Iran). Duplicated glucose level of $\geq$6.1 mmol L^{-1} or $\geq$110 (mg dL^{-1}) was used as a criteria for diabetes mellitus. Total cholesterol, TG, Low density lipoprotein (LDL) cholesterol, High Density Lipoprotein (HDL) cholesterol were determined by calorimetric enzymatic technique (ParsAzmun Co. Kits, Karaj,Iran). Creatinine was measured using Jaffe reaction method (ParsAzmun Kits, Karaj, Iran). Activated Partial Thromboplastin Time (APTT) and Prothrombin Time (PT) were assessed using Instrumentation Laboratory (IL) ACL 8000 coagulation analyzer (Beckman Coulter; Fullerton, California) using HemosIL APTT-SP Kit and PT-Fibrinogen HS PLUS kit, respectively. HemosIL von Willebrand factor antigen was used for quantitative determination of von Willebrand factor antigen in human citrated plasma using ACL 8000 coagulation analyzer (Beckman Coulter). Fibrinogen concentration in citrated plasma was measured using ACL 8000 coagulation analyzer (Beckman) using PT-Fibrinogen HS PLUS kit., furthermore specific factor activity of II, V, VII , VIII, IX, X, XI in citrated plasma using HemosIL factor II, V, VII, VIII, IX, X, IX deficient plasmas, respectively using IL ACL 8000 coagulation analyzer (Beckman Coulter) were assessed. All parameters of PLT, MPW, PDW were measured by SYSMAX KX-N21 hematology analyzer. All experimental procedures involving human participants were conducted with due attention to the guidelines approved by the research ethical committee of Chamran university.

Statistical analysis: The results were statistically analyzed. Analysis of distribution was made using the Kolmogoroff-Smirnoff test. Since this test confirmed that all parameters have normal distribution so no additional tests e.g. Mann-Whitney U test, were not necessary and hence the significance of differences were analyzed using t test. Statistical differences in demographical, biochemical and hematological parameters between NIDDM and normal control groups were determined by independent t-test. Statistical analysis was performed using the Statistical Package for the Social Science (SPSS-PC, version 15. SPSS, Inc., Chicago, IL). Missing data proportion was maximally 7.5% in all coagulation factors which not affects the statistic results. Differences between means of coagulation factors were considered significant at $p<0.05$.

RESULTS

Table 1 show the demographic and biochemical parameters of NIDDM patients and normal controls. Fasting blood sugar, TG and systolic pressure in NIDDM patients group were statistically significantly higher than the group of healthy subjects (Table 1). As it evident there are no significant differences in age, sex, Body Mass Index (BMI) as well as in total cholesterol, HDL, LDL, PLT, PDW and MPV between patient and healthy group. The Kolmogoroff-Smirnoff goodness-of-fit test is applied to check the normality of coagulation factors measured in the present study (Table 2). The normal distribution of the data is confirmed at significance levels of 0.05. Table 3 summarizes the specific activity or concentration of some coagulation factors and Table 4 contains the results of coagulation tests for NIDDM and control groups. The concentration of factor I (fibrinogen) and specific activity of factors vWF, II, VII, IX, X and XI show increased amounts for NIDDM patients in comparison with controls group. Instead the specific activity of FV and FVIII show decreased levels in diabetic patients (Table 3). However, only the increase in specific activity of factors II, IX, XI and decrease in activity of factor VIII are statistically significant (p-value<0.05) (Table 3). As depicted in Table 3, there is not considerable increase in fibrinogen concentration in NIDDM (3.71 g lit^{-1}) compare to control (3.58 g lit^{-1}) group. The striking increase in FII or thrombin activity in NIDDM group with the value of 101.58% from its normal level of 94.78% in control group propound it as participant risk factor in hypercoagulable state of diabetes mellitus. Even though the mean activity of FV (87.72%), FVII (97.32%) and VWF (107.03%) in NIDDM

Table 1: Demographic and biochemical characteristics of NIDDM patients and healthy controls:

Parameter	Diabetics n = 40	Control n = 21	Significance
Gender (% male)	50.00	52.00	Ns
Mean age (years)	54.10±1.58	51.66±2.11	Ns
BMI (kg m^{-2})	28.65±0.89	25.42±0.36	p<0.01
Total Cholesterol (mg dL^{-1})	201.33±6.66	187.53±7.53	Ns
TG (mg dL^{-1})	227.76±17.79	147.46±24.3	p<0.05
FBS (mg dL^{-1})	166.46±13.64	106.66±15.43	p<0.01
Serum Creatinine (μmol L^{-1})	1.00±0.04	0.95±0.06	Ns
HDL (mg dL^{-1})	59.96±1.92	58.40±4.32	Ns
LDL (mg dL^{-1})	95.80±4.50	93.03±8.18	Ns
Systolic blood pressure (mmHg)	132.50±2.01	117.00±1.52	p<0.001
PLT (10^{+3} μL^{-1})	261.76±10.55	255.06±18.29	Ns
MPV (fl)	9.78±0.16	9.49±0.30	Ns
PDW (fl)	12.58±0.28	12.46±0.69	Ns

Ns: Non significant

Table 2: Normality test of Kolmogoroff-Smirnoff for coagulation factors, a: in NIDDM

	Kolmogoroff-Smirnoff		
Factors	Statistic	df	Significance
NIDDM			
Fibrinogen	0.090	39	0.200
Factor II	0.105	39	0.200
Factor V	0.129	39	0.093
Factor VII	0.125	39	0.119
Factor VIII	0.197	39	0.097
VWF	0.087	39	0.200
Factor IX	0.097	39	0.200
Factor X	0.093	39	0.200
Factor XI	0.200	39	0.056
Controls			
Fibrinogen	0.953	19	0.096
Factor II	0.654	19	0.057
Factor V	0.943	19	0.054
Factor VII	0.928	19	0.058
Factor VIII	0.904	19	0.093
VWF	0.966	19	0.286
Factor IX	0.940	19	0.072
Factor X	0.970	19	0.385
Factor XI	0.936	19	0.244

Table 3: Coagulation factors concentration in NIDDM and normal controls. Independent t-test parameters are shown

	Mean			
Coagulation factor	Patient (40)	Control (21)	t-value	p-value
Fibrinogen (gr/liter)	3.71	3.58	0.65	0.258
II (%)	101.58	94.78	1.30	0.010
V (%)	87.72	96.31	0.87	0.194
VII (%)	97.32	93.46	1.39	0.085
vWF (%)	107.037	96.58	1.15	0.126
VIII (%)	59.22	92.17	1.86	0.034
IX (%)	111.86	88.23	2.94	0.004
X (%)	106.63	99.46	2.75	0.082
XI (%)	103.00	83.60	2.94	0.002

Table 4: Coagulation test for NIDDM and normal controls. Independent t-test parameters are shown

	Mean			
Coagulation test	Patient (40)	Control (21)	t-value	p-value
APTT (seconds)	34.81	29.79	1.31	0.001
PT (seconds)	12.48	12.39	0.18	0.861
PTINR	0.97	0.94	0.36	0.715

group are differ from their respective activity in control group of 96.31, 93.46 and 96.58 but their difference are not statistically significant and their involvement in accelerated thrombotic events is doubtful. The next two markedly altered factors are FVIII and FIX. Present results show that

FVIII decreased considerably (p = 0.034) from 92.17% of control group to 59.22% in NIDDM group. This decrement could be taken as beneficiary parameter for life saving in NIDDM patients. FIX instead shows increase pattern from 88.23% of control group to 111.86% in NIDDM group with p-value of 0.004. FIX with half-life of 1-3 days activating FX through intrinsic pathway of coagulation cascade makes another threatening source for thrombotic complications. The mean activity of FX in NIDDM show increased activity of 106.63% in contrast to control group of 99.46%. Although the increase in FX is not significant but the p-value of 0.082 put it at the edge of being risk factor for vascular abnormality. The last coagulation factor in Table 3 is FXI which shows significant increase in NIDDM group of 103% in contrast to control group of 83.6% with p-value of 0.002. FXI itself is making another risk factor for vascular events via intrinsic pathway of coagulation cascade. Table 4 shows the results for coagulation test including APTT, PT and PT_{INR} tests for NIDDM and control groups. Significantly (p-value = 0.001) prolonged APTT for NIDDM group of 34.81 sec against 29.79 sec of control group show the decreased activity of intrinsic and common pathway of coagulation. This prolongation of thromboplastin time may exerted by decreased activity of pathways cofactors, i.e., cofactor V and VIII in common and intrinsic pathway, respectively. Factor XI or Plasma Thromboplastin Antecedent (PTA) in intrinsic pathway significantly increased (p-value = 0.002) in diabetic patients (103%) in contrast to control group (83.60%). Prothrombin time (PT) and its normalized international value (PT_{INR}) results are shown in Table 4. PT and PT_{INR} are used as an index for the involvement of extrinsic and common pathways of coagulation. Present results show no significant changes in this two coagulation tests. These two and all above result were in agreement with our theory about the less involvement of extrinsic pathway in hypercoagulability state in diabetic conditions.

DISCUSSION

Diabetes mellitus is a heterogeneous disease affecting metabolism of various compounds including carbohydrates, lipids and proteins and also impairs biological processes such as coagulation homeostasis which cause vascular thrombotic problems (Hameed *et al*., 2002; Carr, 2001). These problems are predominantly manifested as accelerated coagulation, vascular complications and ultimately as serious problems such as cardiovascular disease and myocardial infarction. The kind and the extent of diabetic complications and their severities are depends on the duration of diabetes onset, dietary regime, physical activity and the treatment protocol taken by patients. From this point of view diabetics could not be categorized in to homogeneous groups easily and it is hard to make a reasonable comparison between the groups in order to obtain reliable results, so we hypothesized that fact itself helps to understand the origin of conflicting reports about the pattern and the significance of coagulation factors change in NIDDM patients as described in details in the introduction part (Collier *et al*., 1992; Carmassi *et al*., 1992; Kluft and Jespersen, 2002; Wakabayashi and Masuda, 2002; Bolaman *et al*., 2007; Schneider *et al*., 2005; Madan *et al*., 2010). Accordingly and based on our findings, we conclude that NIDDM conditions depends to its onset time and treatment history impose vast disturbance in coagulation factors turn over, mainly manifested as hypercoagulable state threatening diabetics life. Table 1 outlined the demographic and biochemical data of NIDDM and control groups. These data show increased BMI, fasting blood sugar, blood triglycerides and blood pressure which are characteristics of type II diabetes mellitus are seen in NIDDM groups. These findings are in agreement with previous reports (Frankel *et al*., 2008; Barillari *et al*., 2009). Table 2 include the result of normality test of Kolmogoroff-Smirnoff done for the data obtained for coagulation factors. The confirmed normality of data distribution

permits further statistical analysis. Present results of determination of plasma coagulation are depicted in Table 3. Table 3 shows that FIX activity is markedly increased in NIDDM group. FIX is a serine protease enzyme in intrinsic pathway of coagulation converts FX to it's activated, FXa, in the presence of FVIII, calcium ion and phospholipid membrane. Hence increased levels of FIX in NIDDM group make a threatening factor for cardiovascular disease through intrinsic pathway. As depicted in Table 3 the activity of FXI is also increased considerably in NIDDM, from 83.60 to 103% ($p = 0.002$), making another risk factor for vascular complication as were seen in patient with cardiovascular disease (Merlo *et al*., 2002; Minnema *et al*., 2000). FXI by activating FIX triggers the intrinsic pathway of coagulation cascade. Factor X is a vitamin K dependent serine protease with 2-2.5 days of half-life, converting inactive factor II (prothrombin) to its active thrombin or IIa form. Factors II and X are the enzymes acting together in common pathway of coagulation. Activated factor II or thrombin is a vitamin K dependent serine protease with 2-4 days of half-life, converts fibrinogen to its active form fibrin. Generally our findings indicate that intrinsic pathway is more responsible for hypercoagulable state than extrinsic pathway because more increment in factors IX and XI in NIDDM than FX and FII. As shown in Table 4 from coagulation tests done in this work only the APTT test is significantly changed in NIDDM group. Unlike what expected from the hypercoagulable state induced by diabetes disease. The APTT test shows prolonged coagulation time from 29.79 sec in control group to 34.81 sec in NIDDM group. We hypothesize that the decrease in mean activity of FV and FVIII activities is the correspondent cause for the prolonged coagulation time and prolonged APTT in NIDDM could considered as compensation mechanism in diabetic patients to save their life against precocious cardiovascular disease. However, mark increase in cytosolic pressure, fasting plasma glucose and plasma cholesterol remain as additional risk factors in NIDDM patients. Overall we think that there is no evidence for initiation of thrombotic problems and microthrombosis by hypercoagulable state alone, instead we think there should be other triggers for thrombosis formation such as the presence of vascular damage and other risk factors. Base on present finding and previous studies, since thrombotic complications and hypercoagulable state in NIDDM patients are introductions to vascular and cardiovascular complications, as a prognostic clue the simultaneous measurement of specific activity of factors II, IX, XI and APTT in NIDDM patients could be initially prescribed, since these factors show critical and more specific alterations in early stages of NIDDM disease and gives awfully bad prognosis coagulation disturbance leading to vascular complications, therefore hypercoagulable state management may have preventive value in subsequent vascular complication in this patients.

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REFERENCES

Barillari, G., E. Favor, S. Pasca and E. Bigotto, 2009. Coagulation and oxidative stress plasmatic levels in a type 2 diabetes population. Blood Coagul. Fibrinolysis, 20: 290-296.

Behnam-Rassouli, M., M.B. Ghayour and N. Ghayour, 2010. Microvascular complications of diabetes. J. Biol. Sci., 10: 411-423.

Berliner, J.I., A.C. Rybicki, R.C. Kaplan, E.S. Monrad, R. Freeman and H.H. Billett, 2002. Elevated levels of Factor XI are associated with cardiovascular disease in women. Thrombosis Res., 107: 55-60.

Bolaman, Z., F. Kok, G. Kadikoylu, N. Kiylioglu, E. Guney and A. Akyol, 2007. The changes of coagulation parameters and microvascular complications in diabetes mellitus. Endocrinologist, 17: 196-199.

Bonetti, P.O., L.O. Lerman and A. Lerman, 2003. Endothelial dysfunction: A marker of atherosclerotic risk. Arterioscler. Throm. Vasc. Biol., 23: 168-175.

Borissoff, B.J.I., S. Heeneman, E. Kilinç, P. Kassak and R. Van Oerle *et al.*, 2010. Early atherosclerosis exhibits an enhanced procoagulant state. Circulation, 122: 821-830.

Bots, M.L., P.C. Elwood, J.T. Salonen, A.F. De Concalves and J. Sivenius *et al.*, 2002. Level of fibrinogen and risk of fatal and non-fatal stroke. Eurostork: A collaborative study among research centres in Europe. Epidemiol. Commun. Health., 56: 14-18.

Cardigan, R.A., H. McGloin, I.J. Mackie, S.J. Machin and M. Singer, 1999. Activation of the tissue factor pathway occurs during continuous venovenous hemofiltration. Kidney Int., 55: 1568-1574.

Carmassi, F., M. Morale, R. Puccetti, F. De Negri, F. Monzani, R. Navalesi and G. Mariani, 1992. Coagulation and fibrinolytic system impairment in IDDM. Thromb Res., 32: 680-684.

Carr, M.E., 2001. Diabetes mellitus: A hypercoagulable state. J. Diabetes Complications, 15: 44-54.

Collier, A., A. Rumiey, A.G. Rumley, R.P. John, P.L. John, D.O. Gordon and S. Micheal, 1992. Free radical activity and hemostatic factors in NIDDM patients with and without microalbuminuria. Diabetes, 41: 909-913.

Dubrey, S.W., D.R. Reaveley, M. Seed, D.A. Lane and H. Ireland *et al.*, 1994. Risk factors for cardiovascular disease in IDDM: A study of identical twins. Diabetes, 43: 831-835.

El-Hagracy, R.S., G.M. Kamal, I.M. Sabry, A.A. Saad, N.F.A. El-Ezz and H.A.R. Nasr, 2010. Tissue factor, tissue factor pathway inhibitor and factor VII activity in cardiovascular complicated type 2 diabetes mellitus. OMJ, 25: 173-178.

El-Khawand, C., J. Jamart, J. Donckier, B. Chatelain, E. Lavenne, M. Moriau and M. Buysschaert, 1993. Hemostasis variables in type I diabetic patients without demonstrable vascular complications. Diabetes Care, 16: 1137-1145.

Elhadd, T.A., T.A. Abdu, J. Oxtoby, G. Kennedy and M. McLaren *et al.*, 2001. Biochemical and biophysical markers of endothelial dysfunction in adults with hypopituitarism and severe GH deficiency. J. Clin. Endocrinol. Metab., 86: 4223-4232.

Eteng, M.U., B.J. Bassey, I.J. Atangwho, G.E. Egbung, E.U. Eyong, P.E. Ebong and A.O. Abolaji, 2008. Biochemical Indices of macrovascular complication in diabetic rat model: Compared effects of *Vernonia amygdalina*, *Catharantus roseus* and chlorpropamide. Asian J. Biochem., 3: 228-234.

Falati, S., P. Gross, G. Merrill-Skoloff, B.C. Furie and B. Furie, 2002. Real-time *in vivo* imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. Nat. Med., 8: 1175-1180.

Frankel, D.S., J.B. Meigs, J.M. Massaro, P.W.F. Wilson, C.J. O'Donnell, R.B. D'Agostino and G.H. Tofler, 2008. Von willebrand factor, type 2 diabetes mellitus and risk of cardiovascular disease. Circulation, 118: 2533-2539.

Hajifathali, A., K. Bahram, P. Aliakbar, R.L. Ghasem and C. Rouzbeh, 2005. Coagulation factor V mutations in iranian patients with venous thrombosis. Pak. J. Biol. Sci., 8: 1272-1274.

Hameed, A., S.A. Malik, F. Rabbi, A. Sharif and N. Ahmad *et al.*, 2002. Diabetic complications: Influence of age, sex, family history, duration, glycemic control and obesity. J. Biol. Sci., 2: 710-714.

Harris, P.L., D.R. Harvey and B.P. Bliss, 1978. The importance of plasma lipid, glucose, insulin and fibrinogen in femoropopliteal surgery. Br. J. Surg., 65: 197-200.

Ibbotson, S.H., D. Walmsley, J.A. Davies and P.J. Grant, 1992. Generation of thrombin activity in relation to factor VIII: C concentrations and vascular complications in type 1 (insulin-dependent) diabetes mellitus. Diabetologia, 35: 863-867.

Karami, H., K. Vahidshahi, M. Kosarian, H. Karami and S. Shahmohammadi *et al.*, 2010. Assessment of coagulation state and its related factors in thalassemia intermedia patients referred to thalassemia research center at booali sina hospital sari/IR Iran in 2007. Pak. J. Biol. Sci., 13: 448-451.

Kessler, L., M.L. Wiesel, P. Attali, J.M. Mossard, J.P. Cazenave and M. Pinget, 1998. Von willebrand factor in diabetic angiopathy. Diabetes Metab., 24: 327-336.

Kluft, C. and J. Jespersen, 2002. Diabetes as a procoagulant condition. Br. J. Diabetes Vascular Dis., 2: 358-362.

Koster, T., A.D. Blann, E. Briet, J.P. Vandenbroucke and F.R. Rosendaal, 1995. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet., 345: 152-155.

Kucharska-Newton, A.M., D.J. Couper, J.S. Pankow, R.J. Prineas and T.D. Rea *et al.*, 2009. Hemostasis, inflammation and fatal and nonfatal coronary heart disease. Arteriosclerosis, Thrombosis, Vascular Biol., 29: 2182-2190.

Laakso, M. and S. Lehto, 1998. Epidemiology of risk factors for cardiovascular disease in diabetes and impaired glucose tolerance. Atherosclerosis, 137: 65-73.

Lee, A.J., F.G. Fowkes, A. Rattray, A. Rumley and G.D. Lowe, 1996. Haemostatic and rheological factors in intermittent claudication: The influence of smoking and extent of arterial disease. Br. J. Haematol., 92: 226-230.

Li, L., T. Sawamura and G. Renier, 2003. Glucose enhances endothelial LOX-1 expression: Role for LOX-1 in glucose-induced human monocyte adhesion to endothelium. Diabetes, 52: 1843-1850.

Lip, G.Y. and A. Blann, 1997. Von Willebrand factor: A marker of endothelial dysfunction in vascular disorders? Cardiovasc. Res., 34: 255-265.

Lohmann, C., N. Schafer, T. Von Lukowicz, M.A.S. Stein and J. Boren *et al.*, 2009. Atherosclerotic mice exhibit systemic inflammation in periadventitial and visceral adipose tissue, liver and pancreatic islets. Atherosclerosis, 207: 360-367.

Lowe, G.D., M.M. Drummond, A.R. Lorimer, G.D. Lowe and M.M. Drummond *et al.*, 1980. Relation between extent of coronary artery disease and blood viscosity. BMJ, 280: 673-674.

Lusis, A., 2000. Atherosclerosis. Nature, 407: 233-241.

Mackman, N., 2004. Role of tissue factor in hemostasis, thrombosis and vascular development. Arterioscler Thromb Vasc. Biol., 24: 1015-1022.

Madan, R., B. Gupta, S. Saluja, U.C. Kansra, B.K. Tripathi and B.P. Guliani, 2010. Coagulation profile in diabetes and its association with diabetic microvascular complications. J. Assoc. Physicians India, 58: 481-484.

Makin, A.J., A.D. Blann, N.A.Y. Chung, S.H. Silverman and G.Y.H. Lip, 2004. Assessment of endothelial damage in atherosclerotic vascular disease by quantification of circulating endothelial cells. Relationship with von Willebrand factor and tissue factor. Eur. Heart J., 25: 371-376.

Meade, T.W., J.A. Cooper, Y. Stirling, D.J. Howarth, V. Ruddock and G.J. Miller, 1994. Factor VIII, ABO blood group and the incidence of ischaemic heart disease. Br. J. Haematol., 88: 601-607.

Meade, T.W., S. Mellows, M. Brozovic, G.J. Miller and R.R. Chakrabarti *et al.*, 1986. Haemostatic function and ischaemic heart disease: Principal results of the northwick park heart study. Lancet, 2: 533-537.

Meigs, J.B., C.J. O'Donnell, G.H. Tofler, E.J. Benjamin and C.S. Fox *et al.*, 2006. Hemostatic markers of endothelial dysfunction and risk of incident type 2 diabetes: The framingham offspring study. Diabetes, 55: 530-537.

Merlo, C., W.A. Wuillemin, M. Redondo, M. Furlan and I. Sulzer *et al.*, 2002. Elevated levels of plasma prekallikrein, high molecular weight kininogen and factor XI in coronary heart disease. Atherosclerosis, 161: 261-267.

Minnema, M.C., R.J. Peters, R. De Winter, Y.P.T. Lubbers and S. Barzegar *et al.*, 2000. Activation of clotting factors XI and IX in patients with acute myocardial infarction. Arterioscler Thromb Vasc Biol., 20: 2489-2493.

Ray, S.A., M.R. Rowley, A. Loh, S.A. Talbot, D.H. Bevan, R.S. Taylor and J.A. Dormandy, 1994. Hypercoagulable states in patients with leg ischaemia. Br. J. Surg., 81: 811-814.

Reverter, J.L., J.C. Reverter, D. Tàssies, F. Rius and J. Monteagudo *et al.*, 1997. Thrombomodulin and induced tissue factor expression on monocytes as marker of diabetic microangiopathy: A prospective study on hemostasis and lipoproteins in insulin dependent diabetes mellitus. Am. J. Hematol., 56: 93-99.

Rezaeian, Z., G. Torkaman, F. Nad-ali, R. Ravanbod and A.A. Pourfathollah *et al.*, 2006. Effect of physical fitness on the coagulant activity of healthy young men. Pak. J. Biol. Sci., 9: 2032-2039.

Schneider, D.J., 2005. Abnormalities of coagulation, platelet function, and fibrinolysis associated with syndromes of insulin resistance. Coronary Artery Dis., 16: 473-476.

Thor, M., A. Yu and J. Swedenborg, 2002. Markers of inflammation and hypercoagulability in diabetic and nondiabetic patients with lower extremity ischemia. Thrombosis Res., 105: 379-383.

Tripodi, A., A. Branchi, V. Chantarangkul, M. Clerici, G. Merati, A. Artoni and P.M. Mannucci, 2010. Hypercoagulability in patients with type 2 diabetes mellitus detected by a thrombin generation assay. J. Thromb Thrombolys, 31: 165-172.

Vaidyula, V.R., G.J. Criner, C. Grabianowski and A.K. Rao, 2009. Circulating tissue factor procoagulant activity is elevated in stable moderate to severe chronic obstructive pulmonary disease. Thrombosis Res., 124: 259-261.

Voetsch, B. and J. Loscalzo, 2004. Genetic determinants of arterial thrombosis. Arteriosclerosis Thromb. Vasc. Biol., 24: 216-229.

Wakabayashi, I. and H. Masuda, 2002. Relation of serum sialic acid to blood coagulation activity in type 2 diabetes. Blood Coagulation Fibrinolysis, 13: 691-696.

Weiss, C., S. Regele, T. Velich, P. Bartsch and T. Weiss, 2000. Hemostasis and fibrinolysis in patients with intermittent claudication: Effects of prostaglandin E1. Prostaglandins, Leukotrienes Essent Fatty Acids, 63: 271-277.

Whiteley, W., W.L. Chong, A. Sengupta and P. Sandercock, 2009. Blood markers for the prognosis of ischemic stroke: A systematic review. Stroke, 40: 380-389.

Wiseman S., G. Kenchington, R. Dain, C.E. Marshall, C.N. McCollum, R.M. Greenhalgh and J.T. Powell, 1989. Influence of smoking and plasma factors on patency of femoropopliteal vein grafts. Br. Med. J., 299: 643-646.

Woodburn, K.R., G.D. Lowe, A. Rumley, J. Love and J.G. Pollock, 1995. Relation of haemostatic, fibrinolytic and rheological variables to the angiographic extent of peripheral arterial occlusive disease. Int Angiol., 14: 346-352.

Zachary, T. and M.D. Bloomgarden, 2011. Diabetes and cardiovascular disease. Diabetes Care, 34: 24-30.