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Original Article

Postprandial phase fluctuations can trigger the coagulation cascade

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Abstract: Background and aims: Cardiovascular Diseases (CVD) are the most common causes of mortality and morbidity among patients with type 2 diabetes. Poorly controlled postprandial hyperglycemia contributes to the development of atherosclerosis. Fluctuations of the postprandial glucose levels bring changes in the coagulation system and propensity to thrombosis. Our aim was to determine the change of plasma coagulation parameters like D-Dimer, P-Selectin, Plasminogen activator inhibitor-1 (PAI-1), Prothrombin fragments 1-2 (PTF 1-2) in comparison to the fasting levels in 15 healthy controls and type 2 diabetic patients under treatment of various agents (metformin, insulin secretagog agents and insulin). Materials and methods: Blood samples were withdrawn after 12 h of fasting (min 0) and following breakfast composed of foods proper for each person, at 60th, 90th and 120th minutes. Fasting and 60th, 90th, and 120th minute measurements of glucose, insulin, triglyceride, D-Dimer, P-Selectin, PAI-1, PTF 1-2 had been performed. HA1C and fructosamine were measured also. Results: Some coagulation parameters tend to be changed at the postprandial phase in diabetics as well as in healthy controls. At the postprandial phase, PAI-1 increased significantly in both healthy controls and in all groups of diabetics. The fasting levels of fibrinogen, D-Dimer and P-Selectin were high in diabetics in comparison to healthy controls. An increase in the levels of P-Selectin, PAI-1 and PTF 1-2 at the postprandial phase was observed in healthy persons. Patients receiving insulin secretagog therapy showed an increase in the postprandial levels of PAI-1 like healthy controls. Patients receiving metformin showed an increase in the postprandial levels of PAI-1 and PTF 1-2. Postprandial phase changes in patients receiving metformin were similar to healthy controls. Poorly controlled, older patients with longer diabetes duration had been receiving insulin and these mentioned patients' levels of fibrinogen, D-Dimer and P-Selectin were high in the fasting state and showed an increase in PAI-1 at the postprandial phase. Postprandial levels of PTF 1-2 and D-Dimer were high in insulin treated patients. Levels of fibrinogen and D-Dimer were higher in patients with retinopathy. HA1C and fructosamine were correlated with the coagulation parameters like P-selectin, PAI-1 and PTF 1-2 levels. Correlations showed us that not only postprandial hyperglycemia but also accompanying diabetes, obesity, dyslipidemia and hypertension can aggravate this coagulation tendency at the postprandial phase. Conclusion: Postprandial phase changes can trigger postprandial coagulation cascade in diabetics as well as healthy persons.

Keywords: Diabetes mellitus, postprandial hyperglycemia, D-Dimer, P-Selectin, plazminogen activator inhibitor-1, prothrombin fragments 1-2, coagulation

Introduction

Changes in human behavior and lifestyle over the last century have resulted in a dramatic increase in the incidence of diabetes worldwide. The epidemic is chiefly of type 2 diabetes [1]. Prevalence of diabetes in adults worldwide was estimated to be 4.0% in 1995 and to rise to 5.4% by the year 2025. It is higher in developed than in developing countries [2]. Diabetes mellitus is a major risk factor for cardiovascular diseases [3]. Diabetes is considered as a "coronary disease equivalent" [4]. Postprandial hyperglycemia is a contributing factor for the development of atherosclerosis. Poorly controlled postprandial phase may influence the onset of diabetic complications [5]. Postprandial phase is characterized with a rapid and large increase in blood glucose levels and this condition is associated with increased cardiovascular diseases (CVD). Hyperglycemia, especially postprandial hyperglycemia causes also altera-

Table 1. Baseline clinical features of the participants in the study

	Control (n=45) Med ± SD Med. 25% 75%	Patients (n=45) Med ± SD Med. 25% 75%	
BMI (kg/m^2)*	25.7±2.92	29.8±5.23	P=0.006 P<0.01
SBP (mm Hg)*	116.4±17.9	118.1±14.9	P=0.498 P>0.05
DBP (mm Hg)*	70.8±10.0	74.3±10.2	P<0.05
Pulse (beat/min)*	85±10.6	83.6±12.8	P=0.457 P>0.05
Meal Consumption Time (min)*	13.4±5.33	11.1±4.8	P=0.118 P>0.05
Glucose 0. min (mg/dl)*	75.7±10.4	120.1±55.7	P=0.003 P<0.001
HbA1c (%)*	4.94±0.36	7.16±2.17	P=0.000 P<0.001
Fructoseamine (umol/L)*	228±13.7	302±77.8	P=0.000 P<0.001
TG 0. min (mg/dl)**	126.7±57.6 119.00 (83.00-175.00)	168.4±78.1 145.50 (123.75-202.75)	P=0.062 P>0.05
HDL (mg/dl)*	50.86±15.34	42.71±7.98	P=0.010 P<0.01
C-pep-0. min (ng/ml)*	2.53±0.51	2.72±2.01	P=0.266 P>0.05
C-pep-60. min (ng/ml)*	6.97±2.64	7.37±2.7	P=0.627 P>0.05
Insulin 0. min (uU/ml)**	5.98±1.96 6.08 (4.29-7.76)	11.5±13.82 8.71 (4.40-11.80)	P=0.044 P<0.05
CRP (ng/dl)**	0.34±0.19 0.33 (0.18-0.46)	0.43±0.32 0.33 (0.19-0.56)	P=0.185 P>0.05
Platelets* (10^3/ul)	248.60±41.2	268.11±78.0	P=0.223 P>0.05
MPV (fl)*	8.68±1.03	8.78±1.12	P=0.156 P>0.05
Fibrinogen (mg/dL)*	296.26±54.51	351.73±69.69	P=0.002 P<0.01
D-Dimer 0. min (ug/dL)*	151.95±101.79	377.85±1134.78	P=0.194 P>0.05
P-Selectin 0. min (ng/ml)**	22.61±5.87 21.00 (18.00-27.60)	30.47±15.41 25.90 (20.00-32.00)	P=0.028 P<0.05
PAI-1 (ng/ml)**	8.97±6.45 6.20 (3.20-13.60)	11.12±9.16 7.9 (5.95-12.45)	P=0.286 P>0.05
PTF 1-2 (pmol/L)**	28.37±10.56 25.40 (19.20-33.40)	33.68±26.82 22.15 (18.70-27.62)	P=0.752 P>0.05

*Student t Test; **Mann-Whitney U Test.

tions in the coagulation system and an overproduction of thrombin. During experimental hyperglycemia diabetic subjects as well as normal subjects demonstrated a shortening in the

Table 2. Baseline levels of the groups

	a. Group (Control)	b. Group (Insulin secretagog)	c. Group (Metformin)	d. Group (Insulin)	P
	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	
Age (year)*	47.533±11.357	55.533±7.633	53.933±9.145	56.933±7.459	a, d; P<0.05
BMI (kg/m^2)**	25.718±2.923	32.153±4.376	27.473±3.126	29.831±6.740	a, b
	26.750 (22.970-27.667)	31.900 (29.100-34.500)	28.000 (24.400-29.000)	28.800 (27.300-35.275)	P<0.05
DM duration (year)*		8.500±5.967	4.367±4.116	10.500±6.647	c, d; P<0.05
Meal consumption time (minute)**	13.467±5.33	12.000±4.52	10.000±4.957	11.400±4.27	0.241
	13.000 (10.000-16.750)	10.000 (10.000-15.000)	10.00 (6.500-11.500)	10.00 (10.00-13.00)	P>0.05
LDL (mg/dl)*	122.6±39.30	112.7±40.74	128.5±38.4	96.0±27.0	0.093; P>0.05
Total Chol (mg/dl)*	198.2±44.2	180.2±49.7	200.3±39.9	162.5±40.1	0.077; P>0.05
HDL (mg/dl)**	50.8±15.3	41.4±6.56	49.2±5.017	37.4±7.4	a, d/c, d
	51.0 (43.000-60.750)	41.0 (36.250-46.000)	49.0 (45.25-52.00)	34.0 (32.25-44.250)	P<0.05
HbA1c (%)**	4.9±0.37	7.365±2.070	5.568±0.756	8.56±2.306	d, a/d, c/b, a/b, c
	5.000 (4.605-5.270)	6.750 (5.768-8.490)	5.220 (5.125-6.037)	8.420 (6.500-10.578)	P<0.05
Fructose amine** (umol/L)	228.6±14.1	308.4±77.43	249.11±17.7	349.6±87.26	d, a/d, c/b, a
	229.00 (217.475-240.000)	291.000 (249.450-321.750)	250.0 (232.50-265.250)	343.00 (286.2-414.975)	P<0.05
Insulin 0th min.** (uU/ml)	5.98±1.96	14.2±18.86	8.83±4.97		0.131
	6.08 (4.29-7.76)	9.61 (3.96-11.8)	8.63 (4.55-11.4)		P>0.05
C-peptide 0th min.** (ng/ml)	2.539±0.530	3.728±2.957	2.827±0.960	1.61±0.932	b, d/c, d
	2.400 (2.320-2.825)	3.130 (2.400-3.900)	2.360 (2.018-3.668)	1.770 (0.716-2.382)	P<0.05
C-peptide-60th min.** (ng/ml)	6.973±2.710	7.920±3.170	6.823±2.213		0.109
	6.43 (4.14-9.15)	7.51 (5.78-10.6)	5.92 (5.2-8.74)		P>0.05
CRP (ng/dl)**	0.34±0.19	0.44±0.22	0.34±0.20	0.52±45	0.458
	0.33 (0.22-0.46)	0.42 (0.23-0.62)	0.28 (0.18-0.52)	0.39 (0.23-0.57)	P>0.05

*One way repeated measures analysis of variance; **Kruskal-wallis one way analysis of variance on ranks.

Table 3. Comparison of basal hematological parameters

	a. Group (Control)	b. Group (Insulin secretagog)	c. Group (Metformin)	d. Group (Insulin)	P
	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	
D-Dimer 0th minute (ug/dL)**	151.95±101.79	145.91±73.18	95.09±45.77	892.54±1900.67	a, d/b, d/c, d
	124.80 (85.750-227.025)	161.80 (82.45-189.35)	90.00 (70.95-110.17)	181.50 (126.82-744.32)	P=0.014
P-Selectin 0th minute (ng/ml)**	22.61±5.87	27.40±6.59	27.09±18.92	36.92±16.67	a, d/c, d
	21.0 (18.25-27.05)	26.60 (21.850-32.00)	24.80 (15.95-29.70)	33.40 (26.80-35.60)	P=0.003
PAI-1 0th minute (ng/ml)**	8.97±6.45	9.25±5.99	10.20±4.64	13.90±13.90	P=0.588
	6.2 (3.5-13.30)	7.80 (5.90-9.55)	8.0 (6.10-13.35)	9.00 (6.40-12.250)	P>0.05
PTF 1-2 0th minute (pmol/L)**	28.37±10.56	39.31±33.61	22.15±12.29	39.58±27.69	P=0.063
	25.40 (19.70-32.90)	24.60 (21.025-48.375)	19.20 (15.00-24.475)	26.70 (20.95-47.075)	P>0.05
Fibrinogen (mg/dL)**	296.26±54.51	338.80±59.12	346.66±67.29	378.73±79.24	a, d
	290.0 (245.0-324.0)	342.0 (312.0-371.0)	336.0 (276.0-404.0)	381.0 (314.0-417.0)	P=0.014
Platelet count** (10^3/ul)	248.60±41.28	264.40±56.85	267.13±87.32	272.80±90.91	P=0.818
	255 (214-288)	272 (226-313)	240 (234-275)	251 (197-310)	P>0.05
MPV (fl)*	8.68±1.03	8.97±0.90	9.20±1.21	9.24±1.06	P=0.461
					P>0.05

*One way repeated measures analysis of variance; **Kruskal-wallis one way analysis of variance on ranks.

Table 4. Fasting and postprandial phase changes in the control group

	1-C-Min 0	2-C-60th min	3-C-90th min	4-C-120th min	P
	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	
Glucose* (mg/dl)	75.7±10.4	94.6±34.8	97.5±26.5	93.1±17.3	1, 3/1, 4
	73 (68.5-81.2)	91 (70-107.7)	92 (82-108.5)	92 (78-102.0)	P<0.05
TG* (mg/dl)	126.7±57.6	156.7±65.7	177.3±69.8	187.3±81.7	1, 4/2, 4/1, 3
	119 (84.7-173.5)	158 (103+206.7)	180 (122.7-220.2)	197 (128-236)	P<0.05
Insulin* (uU/ml)	5.9±1.9	38±24.1	32.9±17.5	27±16.8	1, 2/1, 3/1, 4
	6.08 (4.3-7.7)	32.7 (22.3-49.8)	34.7 (15.8-46.3)	24.8 (14.9-33.1)	P<0.05
C-peptide** (ng/ml)	2.539±0.530	6.97±2.71			1, 2
					P<0.05
D-Dimer (ug/dL)***	151.95±101.7	127.24±90.17	194.02±257.81	111.38±93.24	0.384
					P>0.05
P-Selectin (ng/ml)***	22.61±5.87	24.66±6.33	26.52±8.45	28.06±10.29	1, 4
					0.006
					P<0.01
PAI-1 (ng/ml)***	8.97±6.45	27.09±30.29	35.17±21.19	31.90±27.84	1, 2/1, 3/1, 4
					P<0.001
PTF 1-2 (pmol/L)***	28.37±10.56	32.05±15.76	43.60±42.12	76.26±81.71	1, 4/2, 4
					P=0.024

*Friedman repeated measures analysis of variance on rank; **Paired sample T-Test; ***Univariate analysis of variance.

fibrinogen half-life, an increase in fibrinopeptide A, in fragments of prothrombin, in factor VII and in platelet aggregation. All these data suggests that hyperglycemia causes coagulation activation [6]. Diabetic patients have increased thrombotic tendency due to platelet hyper-reactivity and increased activation of prothrombotic coagulation factors. Fibrinolysis is also decreased diabetics. This procoagulant and hypofibrinolytic condition in addition to platelet hyperreactivity and atheromatous vascular changes predisposes diabetics to increased cardiovascular ischemic events [7].

One of the increased procoagulant factors in diabetes is fibrinogen [8]. Fibrinogen plasma levels are an independent risk factor for CVD [7]. Prothrombin fragments 1+2, which are con-

sidered as a sensitive marker for hypercoagulability and as a reliable marker for thrombin generation has been reported to be elevated in diabetics [8, 9].

There is evidence that enhanced activity of platelets exists in diabetics. Markers of platelet activation, like β-Thromboglobulin, platelet factor 4, thrombaxane B2 and fibronectin are increased in diabetics [9]. Another marker of platelet activation is P-selectin. High P-selectin expression is related with CVD and optimal blood glucose control can restore high P-selectin levels to normal [10].

Interactions between activated platelets and coagulation factors result in a hypercoagulable state and with the formation of fibrin clot. Clot lysis occurs as a result of plasmin formation from plasminogen, which is facilitated by tissue plasminogen activator (tPA) and inhibited by plasminogen activator inhibitor-1 (PAI-1) [11]. PAI-1 has been implicated in human thrombosis. Drugs that lower glucose levels also decrease PAI-1 levels [12]. Another important molecule for fibrinolysis is D-Dimer. D-Dimer is a well established marker used to exclude deep vein thrombosis, but it is also increased in non-thrombotic disorders like diabetes. D-Dimer plasma levels reflect the amount of lysed cross-linked fibrin [13]. The aim of this study was to investigate whether patients with type 2 diabetes show change at the postprandial phase in some parameters of hemostatic and fibrinolytic systems like D-Dimer, P-Selectin, PAI-1 and PTF 1-2.

Materials and methods

45 type 2 diabetic patients were included in the study (27 female, 18 male). Our study patients consisted of three groups; 15 patients on diet or plus metformin 850 mg twice daily, 15 patients using rapid acting insulin analogues, and 15 patients having glinides (as repaglinide 2 mg three times daily). The control group consisted of 15 healthy non-diabetic subjects (9 female, 6 male). All subjects were free from clinically apparent atherosclerotic disease, cerebrovascular accident and/or peripheral vascular disease. Patients with malignancy, history of coagulation disorder, autonomic neuropathy, chronic liver and renal disease, anemia, hypoalbuminemia, and hemoglobinopathy were excluded from the study. Patients using anticoagulant agents, antiplatelet agents and oral contraceptives were also excluded. Patients were maintained on their usual treatment with glinide and rapid acting insulin analogue during the period of blood withdrawal. Retinopathy was diagnosed by an ophthalmologist on the basis of fundoscopic examination. Blood samples were withdrawn after 12 h of fasting (min 0) and following breakfast composed of foods proper for each person, at 60^{th}, 90^{th} and 120^{th} minutes. In the control group blood samples were collected at morning fasting and at 60^{th}, 90^{th} and 120^{th} minutes following a mixed meal breakfast. Fasting and 60^{th}, 90^{th}, and 120^{th} minute measurements of glucose, insulin, triglyceride, D-Dimer, P-Selectin, PAI-1, PTF 1-2 had been performed. HA1C and fructosamine were measured also.

Statistical data analysis was conducted using SPSS Statistics version 15.0 for Windows. *p*-value of less than 0.05 was considered significant.

Results

Clinical features of the participants involved in this study are presented in **Table 1** (Comparison of the baseline features of the diabetic patients with controls). The baseline levels of basal fibrinogen and P-selectin levels were significantly higher in type 2 diabetic patients than the healthy controls. The other parameters of hemostasis at baseline (0^{th} minute) like D-Dimer, PAI-1 and PTF 1-2 levels did not differ between diabetic patients and control subjects. Diabetic patients were more obese than the controls. Systolic blood pressure and pulse did not differ from the controls, but mean diastolic pressure was higher in diabetics. Meal consumption time, basal triglyceride, and basal and 60^{th} minute C-peptide levels were similar in both groups. Glucose, HA1c and fructoseamine levels were higher in the diabetics as expected. HDL cholesterol levels were lower in the diabetic patients. Diabetic patients' basal insulin levels were higher than controls (patients receiving insulin was not included for this comparison). Platelet counts, MPV levels, and CRP levels were similar in both groups.

Comparison of the four groups at the fasting state is presented in **Table 2**. Patients who used insulin were older than the others. Patients who used insulin secretagog therapy

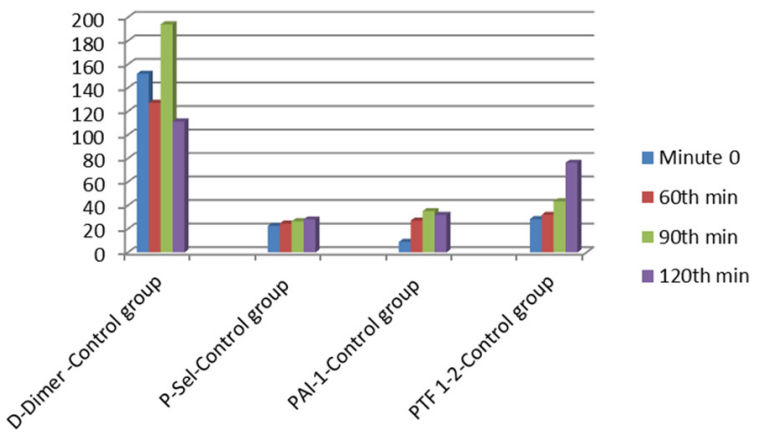

Figure 1. Postprandial changes of the coagulation parametres in the control group.

had the highest BMI. Duration of diabetes was the longest in the insulin using diabetic group. The shortest duration time of diabetes was observed in the patients receiving only metformin. Fibrinogen and D-Dimer levels were significantly higher than to the other groups in the patients who used insulin. Baseline P-selectin was higher in the insulin group in comparison to the control and metformin group. The other parameters at fasting including PAI-1 and PTF 1-2 levels did not differ between groups (**Table 3**).

The glucose level in the control group reached to a peak at the 90th minute. (Postprandial phase changes in the control group have been shown in **Table 4**). Insulin and C-peptide levels reached to a peak at the 60th minute.

D-Dimer levels did not change statistacally significant after meal consumption. P-selectin levels increased postprandial in the control group incrementally. PAI-1 level reached to a peak postprandial at the 90th minute and levels at the 60th minute, 90th minute and 120th minute increased significantly in comparison to baseline. PTF 1-2 levels also increased postprandial in the control group escepially at the 120th minute (**Figure 1**).

Investigation of the parameters in the patients receiving glinide revealed that the glucose level in the glinide group reached to a peak at the 90th minute. (Postprandial phase changes in the glinide group have been shown in **Table 5**).

Triglyceride levels reached to a peak level at the 120th minute in the patients receiving glinide therapy, which was statistically significant. Insulin level reached to a peak level at the 90th minute and was elevated at all post meal times in comparison to basal level. Also C-peptide level increased in comparison to the basal level in patients receiving glinide therapy. D-Dimer level changes were not statistically significant after mixed meal consumption. P-selectin levels increased postprandial in the glinide group incrementally. PAI-1 level reached to a peak postprandial at the 90th minute, where glucose and insulin levels were elevated also. PAI-1 levels at the 60th minute, 90th minute and 120th minute increased significantly in comparison to baseline, but the increase at the 90th minute was much more prominent. PTF 1-2 levels changes were statistically not different in the postprandial phase in the glinide group (**Figure 2**).

The glucose level in the metformin group reached to a peak at the 60th minute. (Postprandial phase changes in the metformin group have been shown in **Table 6**). Insulin and C-peptide levels reached to a peak at the 60th minute in the metformin group. Triglyceride levels reached to a peak level at the 120th minute in the patients receiving metformin therapy which was statistically significant. Insulin level reached to a peak level at the 60th minute and was elevated at 60th and 90th minute post meal times in comparison to basal level.

D-Dimer and P-selectin levels did not change statistacally significant after meal consumption. PAI-1 level reached to a peak postprandial at the 90th minute and these levels increase at the 90th minute was increased significantly in comparison to 60th minute. PTF 1-2 levels also increased incrementally postprandial in the metformin group and the increase at the 120th minute was statistically significant in comparison to baseline (**Figure 3**).

Glucose levels were found to be elevated in the insulin analog using group at the 60th and 90th minute after meal in comparison to fasting state (**Table 7**). Triglyceride levels reached to a

Table 5. Fasting and postprandial phase changes in the glinide group

	1-Glinide-0 min	2-Glinide-60th min	3-Glinide-90th min	4-Glinide-120th min	P
	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	
Glucose* (mg/dl)	132.8±30.1	195.8±61	198.2±70.7	194±67.4	1, 2/1, 3/1, 4 P<0.05
TG* (mg/dl)	170.8±85.4	165.2±83.6	176.3±86.8	185±88.7	1, 4/2, 4 P<0.05
Insulin** (uU/ml)	14.2±18.8 9.6 (4.45-11.8)	36.4±21.1 30.7 (21.7-50.2)	41.7±24.6 36.4 (23.6-57.7)	38.07±24.5 31.3 (19.7-50.2)	1, 2/1, 3/1, 4 P<0.05
C-peptide** (ng/ml)	3.72±2.95	7.92±3.17			1, 2 P<0.05
D-Dimer (ug/dL)****	145.91±73.18	246.44±309.52	371.13±658.89	344.14±709.77	0.447 P>0.05
P-Selectin (ng/ml)****	27.40±6.59	28.65±8.58	31.32±10.07	30.52±7.25	0.162 p>0.05
PAI-1 (ng/ml)****	9.25±5.99	21.96±19.90	36.76±12.25	28.72±10.71	1, 2/1, 3/1, 4/ 2, 3 P<0.001
PTF 1-2 (pmol/L)****	39.31±33.61	71.19±86.71	77.10±109.04	60.56±29.36	P=0.462 P>0.05

*One way repeated measures analysis of variance; **Friedman repeated measures analysis of variance on rank; ***Paired sample T-Test; ****Univariate analysis of variance.

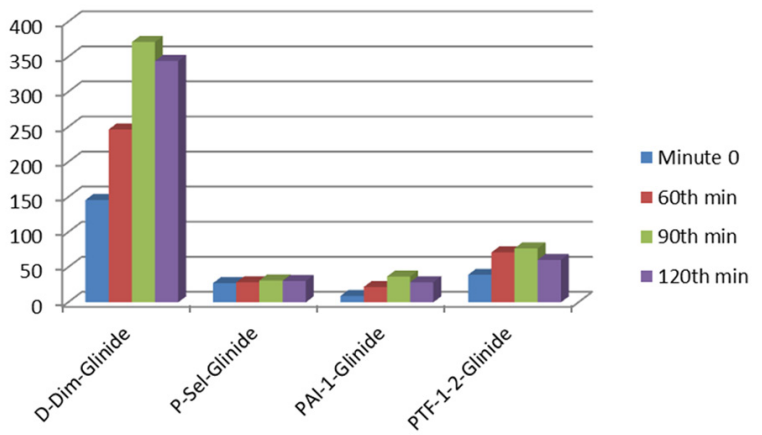

Figure 2. Postprandial changes of the coagulation parameters in the glinide group.

peak at the 120th minute in comparison to basal and 60th minute. D-Dimer, PTF 1-2 and P-selectin levels did not show any significant change in the insulin treated group. PAI-1 levels were elevated during all the postmeal times in comparison to fasting state in the insulin treated group (**Figure 4**).

Discussion

Some studies indicate that the effects of acute hyperglycemia, in particular, postprandial hyperglycemia, have negative effects on the development of diabetic complications [8]. Atherosclerotic vascular disease is common in diabetic individuals. Also studies in people with impaired glucose tolerance and impaired fasting glucose suggest that the pathogenic role of hyperglycemia on the blood vessel wall already exists in the early stages of glucose intolerance. The effect of postprandial hyperglycemia seems to be greater than the effect of fasting blood glucose abnormalities. Based on the results of epidemiological reports, the most appropriate targets in interventional trials are postprandial hyperglycemia or A1C [14]. Postprandial state is a triggering factor for atherosclerosis. The development of cardivascular events like atherosclerosis, myocardial infarction, and sudden cardiac death are connected partly due to increased activity of the coagulation system and thrombus formation. Also several studies describe a relation between plasma concentrations of triglyceride and factor VII coagulant activity. Postprandial hypertriglyceridemia may stimulate the activation of coagulation factor VII and thereby postprandial hy-

Table 6. Fasting and postprandial phase changes in the metformin group

	1-Met-0 min	2-Met-60th min	3-Met-90th min	4-Met-120th min	P
	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	Med. ± SD Med. 25% 75%	
Glucose* (mg/dl)	100.600 ±15.523	155.067±32.003	133.33±34.115	116.467±29.587	1, 2/1, 3/1, 4 2, 3/2, 4/3, 4 P<0.05
TG** (mg/dl)	169.000±55.093 144.0 (131.50-210.25)	168.667±55.006 149.0 (129.75-207.00)	178.133±55.5 166.0 (141.0-201.7)	182.067±58.59 190.0 (135.0-207.25)	2, 3/2, 4 P<0.05
Insulin** (Uu/ml)	8.837±4.978 8.63 (5.06-11.01)	38.160±17.758 30.80 (24.17-52.05)	35.367±13.34 32.20 (23.52-46.55)	26.285±14.763 23.4 (17.15-34.22)	1, 2/1, 3 P<0.05
C-peptide*** (ng/ml)	2.827±0.960	6.823±2.213			1, 2 P<0.05
D-Dimer (ug/dL)****	95.09±45.77	94.34±64.20	148.46±202.05	129.45±150.04	0.577 P>0.05
P-Selectin (ng/ml)****	27.09±18.92	26.94±9.53	25.29±10.18	26.76±7.97	0.938 P>0.05
PAI-1 (ng/ml)****	10.73±4.58	25.89±27.21	41.06±20.33	28.21±16.91	1, 2/1, 3/1, 4/2, 3 P<0.001
PTF 1-2 (pmol/L)****	21.74±12.31	33.25±24.99	33.73±21.36	39.80±2.53	1, 4 P=0.049 P<0.05

*One way repeated measures analysis of variance; **Friedman repeated measures analysis of variance on rank; ***Paired sample T-Test; ****Univariate analysis of variance.

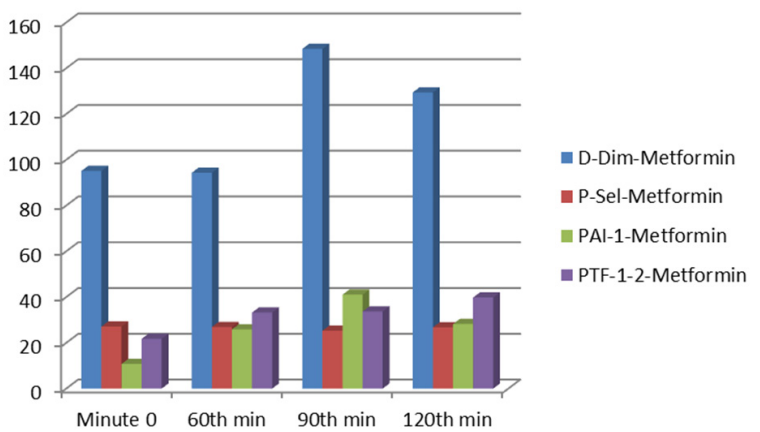

Figure 3. Postprandial changes of the coagulation parametres in the metformin group.

pertriglyceridemia may attract platelet aggregation and thrombotic tendency [8].

We have evaluated D-Dimer, P-Selectin, Plazminogen activator inhibitor-1 (PAI-1), and Prothrombin fragments 1-2 (PTF 1-2) levels at fasting and at the postprandial state. Our study has confirmed that postprandial hyperglycemia is important and the coagulation cascade may be activated in the postprandial state in diabetics, as well as in normal healthy subjects, too. Elevated P-selectin, PAI-1, and PTF 1-2 levels at the postprandial state in diabetics as well as in healthy controls reveals that the coagulation system may be involved after meals like mixed-meals.

In patients with diabetes coagulation factors fibrinogen, factor VII, factor VIII, factor XI, factor XII, kallikrein and von Willebrand factors have been reported to be eleveated [15]. Our patients' fibrinogen levels were higher than the healthy subjects. Fibrinogen has been described as a proven hemostatic factor for increased cardiovascular risk and has been attributed to directly promote atherogenesis [16]. In another study fibrinogen levels were higher among diabetics [17]. Fibrinogen is a cardiovascular risk factor due to inreased thrombin formation. Fibrinogen and thrombin formation have a role in the pathogenesis of atherosclerosis. Also a relationship between fibrinogen and glycemia has been described. [18]. Fibrinogen plasma levels and prothrombin fragments 1+2 are good markers of thrombin generation and D-dimer is marker of fibrin breakdown fragment [19]. Insulin resistance, which is often seen among type 2 diabetics, have been described to be in

Table 7. Fasting and postprandial phase changes in the insulin group

	1-Insulin-0 min	2-Insulin-60th min	3-Insulin-90th min	4-Insulin-120th min	P
	Ort. ± SS Med. 25% 75%	Ort. ± SS Med. 25% 75%	Ort. ± SS Med. 25% 75%	Ort. ± SS Med. 25% 75%	
Glucose* (mg/dl)	126.867±89.334	172.533±81.46	172.80±92.87	160.20±83.51	1, 2/1, 3
	112.0 (66-140.0)	176 (103-225.7)	177 (98.25-225.0)	165 (83.25-242.0)	P<0.05
TG** (mg/dl)	165.533±94.024	167.067±79.745	181.20±84.29	187.53±84.51	1, 4/2, 4
					P<0.05
Insulin** (Uu/ml)	892.54±1900.67	837.02±1721.40	741.72±1511.05	779.41±1447.35	0.299
					P>0.05
D-Dimer (ug/dL)****	67.22±7.07	66.85±6.23	57.47±10.55	44.78±8.93	1, 3/1, 4/2, 3/2, 4/3, 4
					P<0.001
P-Selectin (ng/ml)****	36.92±16.67	37.77±19.82	33.88±8.74	33.90±8.36	0.464
					P>0.05
PAI-1 (ng/ml)****	13.90±13.90	35.49±47.22	34.44±28.90	34.28±33.51	0.028
					1, 2/1, 3/1, 4
					P<0.05
PTF 1-2 (pmol/L)****	36.82±27.98	67.28±58.26	80.69±112.71	85.82±108.27	0.339
					P>0.05

*One way repeated measures analysis of variance; **Friedman repeated measures analysis of variance on rank; ***Paired sample T-Test; ****Univariate analysis of variance.

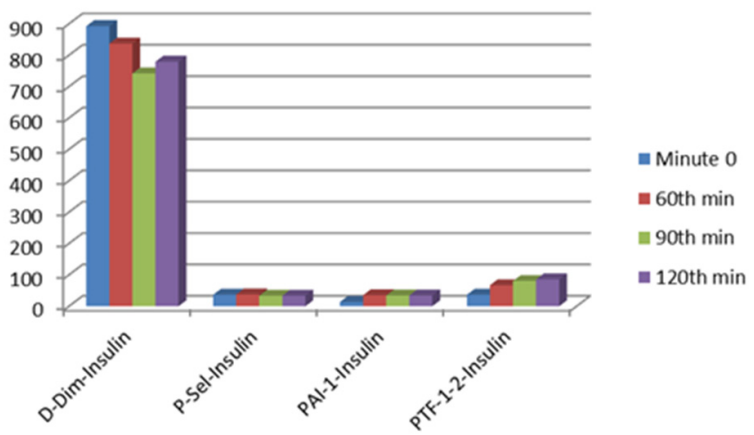

Figure 4. Postprandial changes of the coagulation parameters in the insulin analog group.

association with increased fibrinogen levels [18]. McBane and collagues have found an correlation between age and fibrinogen in type 2 diabetics [20]. Our study has confirmed also a positive correlation between age and fibrinogen levels. Also a correlation between HA1c levels and fibrinogen, and a correlation between fructosamine and fibrinogen were observed. Thrombin is an activator of coagulation and primary hemostasis, but measurement of levels of thrombin is difficult due to rapid inhibition by antithrombins. Therefore indirect measurements of thrombin generation like prothrombin fragments 1-2 are made, which is liberated during the conversion of prothrombin to thrombin [21]. The PTF 1-2 fragments, as a reliable marker of thrombin generation, showed an increase postprandially escepecially at the 120th minute in our study among healthy subjects and in the metformin treated group. Insulin and glinide therapy groups postprandial PTF 1-2 fragments levels were increased also, but these elevations were statistically nonsignificant. These findings suggests that PTF 1-2 levels are elevated postprandially in diabetics as well as in healthy subjects even at the postprandial 120th minute. 120th minute was also time where triglyceride levels also showed a peak. This postmeal increase may be affected by therapy our study shows. It has been shown that this increase may be prevented by red wine consumption with meal, also [22].

P-selectin is another marker of platelet activation. It is secreted from alpha granules of platelets by activation and also expressed on endothelial surfaces. P-selectin levels in diabetic subjects has been reported to be elevated [23]. Our diabetic patients P-selectin levels were

elevated at baseline, also. P-selectin levels were increased postprandially in healthy subjects and in the glinide treated group incrementally, but these changes were not in meaningful ranges statistically. On the other hand insulin and metformin treated groups did not show any significant change, suggesting a beneficial effect for hypercoagulability in this high-risk patient group in terms of postprandial elevation.

Plazminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor and elevated levels of PAI-1 is a marker of impaired fibrinolysis [24]. PAI-1 inhibits serine proteases such as t-PA, and inhibits t-PA associated with clots. Elevated concentrations of PAI-1 is associated with venous thrombosis. Hyperglycemia and hypertriglyceridemia may contribute to elevation of PAI-1 [25].

We have observed that all our diabetic patient groups regardless of the type of therapy showed an increase in the levels of PAI-1 postprandially. The same increase was observed also in healthy patients. The peak time of PAI-1 increase was at the 90th minute in the control group, insülin secretagog group, and in the metformin group. The 120th minute was higher than baseline levels, but this increase was not as high as the 90th minute. Peak increase was observed at the 60th minute in the insulin treated group. 90th and 120th minute levels of PAI-1 in the insulin treatment group was higher to baseline levels. These results suggest that postprandial PAI-1 increase may trigger coagulation among diabetics as well as in healthy subjects.

The other coagulation parameter we have evaluated was D-dimer fragments. D-dimer levels are very useful for the diagnosis of deep vein thrombosis and pulmonary embolism. They are products of fibrin degradation by plasmin, and their plasma levels may reflect a hypercoagulable state and are important for evaluation of fibrinolysis activation [26]. The most prominent finding in order of coagulation parameters in our study was that the D-Dimer levels were high in the insulin group. D-dimer levels in the insulin-receiving group was high at baseline as well as at the postprandial state. The insulin group consisted of more older patients and their diabetes age was higher. An interesting finding was that the D-dimer levels in the metformin treated patients was prominently lower in comparison to other groups. This may suggest that metformin therapy is a good choice of treatment in order of hypercoagulability. Also the longer the diabetes duration means the most problematic patient for hypercoagulabilty. D-dimer levels in the insulin group was higher postprandially in comparison to other groups. The other groups as well as the control group showed a slight increase in D-dimer levels postprandially and this peak was at the 90th minute.

In conclusion, the postprandial state may trigger the coagulation cascade in diabetic patients as well as in healthy persons.

Disclosure of conflict of interest

None.

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