Association of fibrinogen and plasminogen activator inhibitor-1 with diabetes mellitus

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Abstract: As the state of hyperfibrinogenemia in diabetes patients occurs due to hyperglycemia which also activates the coagulative cascade ultimately stimulating hepatic fibrinogen synthesis and thus increases clotting factors and PAI-1 levels in the blood. Therefore, in present study our aim is to correlate between type of diabetes and plasma fibrinogen level and plasminogen activator inhibitor-1. This cross sectional study was conducted at Baqai Medical University (BMU) with the collaboration of Baqai Institute of Diabetology and Endocrinology, Karachi. Data was collected from 161 subjects, out of which 51 were control and 55 were subjects in each type 1 diabetes, type 2 diabetes simultaneously. Anthropometric measurements included measurement of weight, height, BMI and blood pressure which were done for each participant. Blood sugar levels and glycated hemoglobin, lipid profile, PAI-1 and fibrinogen were measured in cases and controls. Out of 161 subjects, 80 (49.7%) were male and 81 (50.3%) were female with mean age of 37.75±1.25 years. Fibrinogen level was significantly decreased in healthy subjects as compared to type 1 and type 2 diabetes subjects P-value<0.0001, however no significant difference was observed in fibrinogen level of type 1 diabetes subjects and type 2 diabetes subjects. Plasminogen activator inhibitor-1 of type 2 diabetes subjects was significantly increased as compared to type 1 diabetes subjects (P-value<0.05) but not significantly different to healthy subjects (P-value>0.05). Since, fibrinogen and plasminogen activator inhibitor type 1 was increased in diabetes patients this predisposed them to increased risk of coronary artery disease, our study further supports the clinical observation that diabetes is a thrombophillic condition.

Keywords: Diabetes mellitus, fibrinogen, plasminogen activator inhibitor-1.

INTRODUCTION

Diabetes is a cluster of dysfunctions characterized by increased blood glucose levels resulting from deficiency of insulin secretion, insulin action and both secretion of insulin and its actions. The continuous state of increased blood glucose levels in diabetes damages the tissues and results in failure of different organs (Abouammoh 2020). The blood clots are formed by a glycopeptide fibrinogen which is also recognized as factor I and it is the common pathway in coagulation cascade (Sobolewski et al, 2018). In type 2 diabetes the plasma fibrinogen level is high which increases cardiovascular morbidity and mortality of diabetes patients (Shemran 2020).

Hyperfibrinogenemia in diabetes is due to increased blood glucose level which causes the activation of coagulation cascade, thus causing thrombin and fibrinogen degradation products formation to increase, that may ultimately stimulate hepatic fibrinogen synthesis. Various standard screening tests of coagulation system function, its Partial thromboplastin time (PT), activated partial thromboplastin time (APTT) and fibrinogen function. its value in monitoring therapeutic anticoagulation is

commonly known (Agarwal 2019).Thus, Diabetes is established as procoagulant disease and causes various micro and macro vascular complications (El-Wakeel et al., 2020).

Plasminogen activator inhibitor-1 (PAI-1) is the member of serpin family of the serine protease inhibitor and it functions as a primary inhibitor, both the urokinase-type (uPA) and tissue-type (tPA) plasminogen activators (Vousden et al., 2020) During impaired fibrinolysis, the levels of plasminogen activator type 1 is used as a biochemical marker and is also known to be associated with reinfarction rates in subjects with previous myocardial infarction (Song et al., 2017). Increased PAI-1 plasma levels are related with risk factors for insulin resistance and Coronary artery disease such as obesity (Liu et al., 2020; Somodi et al., 2018; Recinella et al., 2020) triglycerides (Antunes et al., 2020), insulin (Grandl & Wolfrum 2018) glucose and blood pressure through the rennin angiotensin system (RAS) (Jung et al., 2018; Chen, et al., 2017). Diabetes slows the fibrinolytic system which is the primary means of removing clots due to abnormal clot structure which causes clots to be more resistant to degradation and ultimately causes a spike in plasminogen activator inhibitor-1 levels (El-Wakeel et al., 2017).

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Table 1: Comparison of bio-chemical parameters between healthy subjects, type 1 diabetes and type 2 diabetes subjects

Parameters	Healthy subjects	Type 1 DM subjects	Type 2 DM subjects
N	51	55	55
Gender			
Male	27(52.9%)	25(45.5%)	28(50.9%)
Female	24(47.1%)	30(54.5%)	27(49.1%)
Age (years)	37.69±1.77	23.38±1.11*	52.16±1.37*
BMI (kg/m^2)	27.55±1.25	21.53±0.54*	28.21±0.63*
Systolic blood pressure (mmHg)	111.25±0.64	113.89±2.07	124.52±2.8*
Diastolic blood pressure (mmHg)	72.5±0.76	72.5±1.24	77.98±1.47*
Fasting blood sugar (mg/dl)	88.18±1.06	182.13±13.13*	156.05±9.37*
HbA1c (%)	5.38±0.07	10.2±1.21*	8.42±0.29*
Cholesterol (mg/dl)	189.22±5.98	168.25±4.97*	171.24±6.91
Triglyceride (mg/dl)	127.43±9.66	89.47±5.74*	164.58±14.72
High density lipoprotein (mg/dl)	32.14±0.8	35.73±1.08*	29.07±0.82*
Low density lipoprotein (mg/dl)	127.63±5.39	106.69±4.27*	109.82±5.56*
Very low-density lipoprotein (mg/dl)	25.49±1.93	17.89±1.15*	32.92±2.94
Non-high-density lipoprotein (mg/dl)	157.08±5.89	132.53±4.84*	142.16±6.78

Data presented as mean ± SE; *Significantly different from healthy subjects

Table 2: Correlation of fibrinogen and plasminogen activator inhibitor type 1 with lipid profile of studied subjects

	Fibrinogen		PAI-1	
Parameters	r	P-value	r	p-value
Cholesterol (mg/dl)	-0.061	0.444	0.076	0.335
Triglyceride (mg/dl)	-0.064	0.422	0.057	0.476
High density lipoprotein (mg/dl)	-0.022	0.78	0.056	0.484
Low density lipoprotein (mg/dl)	-0.075	0.343	0.109	0.168
Very low-density lipoprotein (mg/dl)	-0.064	0.422	0.057	0.476
Non-high-density lipoprotein (mg/dl)	-0.062	0.432	0.064	0.421

Spearman's correlation was applied, r: spearman's correlation coefficient, P-value<0.05 considered to be statistically significant

As shown that diabetes is known to increase expression of cell adhesion molecules and it also increases plasminogen activator inhibitor 1 levels; the need for more studies is required to assess the relationship of fibrinogen and PAI-1 with T1DM and T2DM. Therefore, in the present study our aim is to correlate between types of diabetes and plasma fibrinogen level and plasminogen activator inhibitor-1.

MATERIALS AND METHODS

This prospective case-control study was conducted at Baqai Medical University (BMU) with the collaboration of Baqai Institute of Diabetology and Endocrinology (BIDE), Karachi-Pakistan. Ethical approval was obtained from Ethics Committee of the (BMU). The duration of research study was approximately 10 months between January' 2020 to October' 2020. After obtaining verbal and written consent the subjects were included in the study. Participants visiting the outpatient department of BIDE were categorized into three groups; type1 diabetes, type 2 diabetes and healthy controls. Blood samples were obtained for sugar levels, glycated hemoglobin, lipid profile, PAI-1 and fibrinogen levels. The cases included previously diagnosed subjects who were already on diabetes treatment. The controls were without diabetes healthy individuals. Anthropometric measurements included height, weight and blood pressure.

Body mass index was calculated as weight (in kilograms) divided by height (in meters) squared Blood pressure systolic and diastolic was measured by sphygmomanometer. Fasting blood glucose (FBS) was measured by glucose oxidase peroxidase method (GOD PAP method). HbA$_1$c was measured by High Performance Liquid Chromatography (HPLC). Total cholesterol was measured by cholesterol oxidase phenol 4-aminoantipyrine peroxidase (CHOD-PAP method). Triglyceride by glycerine phosphate oxidase peroxidase (GPO-PAP method) and high density lipoproteins (HDL), Low density lipoproteins Cholesterol were measured by direct method. Fibrinogen and plasminogen activator inhibitor type-1 were measured by competitive ELISA method (Enzyme-Linked Immunosorbent Assay) by using ELISA kit.

STATISTICAL ANALYSIS

The Statistical Package for Social Sciences (SPSS) version 20 and excel version 16 were used for statistical analysis. Normality of the data was check by Shapiro-Wilk Test. Spearman's correlation analysis was used to observe the relationship of fibrinogen and plasminogen activator inhibitor-1 with different parameters. Median (IQR) Statistical significance was set at P-value<0.05.

RESULTS

Out of 161 subjects, 55 (34.2%) were type 1diabetes, 55(34.2%) were type 2 diabetes subjects and 51 (31.8%) were healthy controls. Among studied participants, 80 were males (49.7%) and 81 females (50.3%) with mean age of 37.75±1.25 years. The blood pressure both systolic and diastolic was in normal mean range, body mass index was elevated with 25.61 ± 0.53 kg/m^2. Table 1 presents the comparison of bio-chemical parameters between healthy, Type 1 diabetes and Type 2 diabetes subjects. T1DM subjects were statistically significantly different from healthy subjects in FBS, HbA1c and lipid profile parameters (p<0.05). Meanwhile, type 2 diabetes subjects were significantly different in FBS, HbA1c, HDL and LDL levels from healthy subjects.
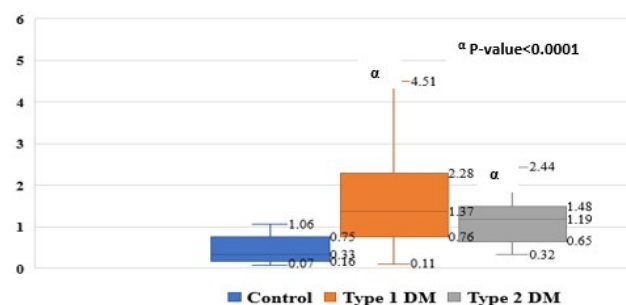
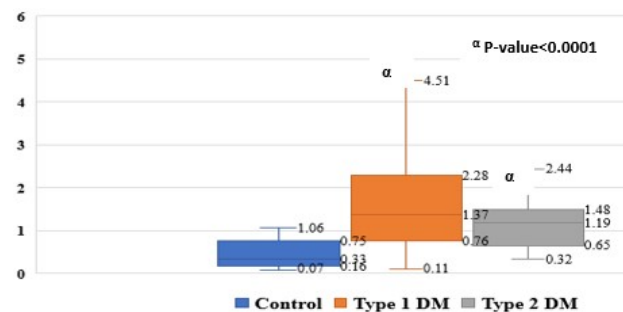$^\alpha$ Significantly different from control

Fig. 1: Comparison of Fibrinogen among studied groups

Table 2 presented correlation of fibrinogen and plasminogen activator inhibitor-1 with lipid profile of studied subjects. No significant correlation of fibrinogen and plasminogen activator inhibitor-1 was observed with any component of lipid profile.

Fig. 1 presented comparison of fibrinogen among studied groups. Levels of fibrinogen in healthy subjects, T1DM and T2DM subjects was found to be (Median (IQR)) 0.33(0.16-0.75), 1.37(0.76-2.28) and 1.19(0.65-1.48) respectively. Fibrinogen was significantly lower in healthy subjects as compared to type 1 and type 2 diabetes subjects (P-value<0.0001) but no significant change was observed in fibrinogen level of type 1 diabetes subjects and type 2 diabetes subjects.

Fig. 2 presented correlation of plasminogen activator inhibitor-1 among studied groups. plasminogen activator

inhibitor-1 in healthy subjects, type 1 diabetes subjects and type 2 diabetes subjects was found to be ((Median (IQR)) 0.83(0.58-1.30), 0.8(0.47-1.19) and 1.07(0.83-1.30) respectively. Plasminogen activator inhibitor-1 of T1DM subjects was significantly higher as compared to T2DM subjects (P-value <0.05) but not significantly different to healthy subjects (P-value >0.05).

$^\beta$ Significantly different from type 1 DM

Fig. 2: Comparison of plasminogen activator inhibitor-1 among studied groups

DISCUSSION

In our study, fibrinogen level was found to be significantly higher in diabetes subjects as compared to in healthy subjects. Shemran *et al*, also found that Plasma fibrinogen level were elevated in diabetes subjects than in the control subjects (Shemran 2020). Similar results were obtained by previous studies where patients with diabetes mellitus had significantly greater fibrinogen level than controls (Kayapinar *et al*., 2019; Lee *et al*., 2017; Somodi *et al.,* 2018) Lee, also showed that in patients with known coronary artery disease, fibrinogen levels were higher than normal subjects. (Lee *et al*., 2017) but in our study, no significant difference was observed in fibrinogen level of T1DM and T2DM subjects. Fibrinogen is recognized as an independent risk factor for developing ischemic heart diseases, hence, regardless of the patients being type 2 or type 1 diabetics; the risk of developing atherosclerosis remain the same.(Shemran 2020).

Plasminogen activator inhibitor-1 of type 2 diabetic subjects was found to be significantly higher in our study as compared to type 1 diabetes subjects but not significantly different to healthy subjects. Somodi also confirms that type 2 diabetes is related with variations in the fibrinolytic system and support the hypothesis that in arterial wall the increased PAI-1 might contribute to the progress of enhanced Coronary vascular disease in Type 2 Diabetes Mellitus. A systemic review was once conducted of 52 epidemiological studies to evaluate the correlation between plasminogen activator inhibitor-1 and Type 2 Diabetes Mellitus, studies reported significantly increased PAI-1 levels between Type2 Diabetes Mellitus individuals, as compared to controls (Dastgheib *et al*

2020) Since, insulin resistance predispose to an increased risk of premature coronary artery disease and mainly the early incidence of myocardial infarction (MI), it is hypothesized that levels of PAI-1 were significantly increased in Type 2Diabetes Mellitus as compared to Type1Diabetes Mellitus which is similar to our study (El-Sayed *et al*., 2018).

A study showing meta-analysis of 31 prospective studies which included data without known cardiovascular diseases and precisely quantified cross-sectional associations of fibrinogen levels with several well known risk factors of metabolic diseases under several variables and proved that fibrinogen levels increases with age which may be because they are indicators of collective atherothrombotic activity (American Journal of Epidemiology, 2007). Whereas, Persil-Ozkan *et al*, found a major positive correlation between tPA levels and BMI, fat-free mass, body weight and waist circumference, and, there was also a significant relationship among BMI and other blood parameters (Persil-Ozkan *et al*, 2019).

Since, in our study no significant correlation of fibrinogen and plasminogen activator inhibitor-1 was observed with any component of lipid profile of studied subjects which is contradictory to Razak *et al.,* who showed progressive association among low density lipoproteins levels, triglycerides levels and serum cholesterol level with plasma fibrinogen levels. Previous study found reverse relation with duration of diabetes and high density lipoproteins level (Razak *et al*, 2019). Furthermore, the plasma TG, LDL-c and total cholesterol concentrations, were also found to be definitely associated with pro-inflammatory PAI-1 levels (Antunes *et al*., 2020). The present study examines the association of levels of fibrinogen and plasminogen activator inhibitor type 1 which are considered as an important inflammatory marker having a pathogenic cause in type 1 as well as type 2 diabetic patients.

CONCLUSION

Fibrinogen and plasminogen activator inhibitor were increased in diabetes patients thus predisposing them to increased risk of coronary artery disease. This study backs the clinical observation that diabetes is a thrombophillic state.

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REFERENCES

Agarwal C, Bansal K, Pujani M, Singh K, Chauhan V, Rana D and Lukhmana S (2019). Association of coagulation profile with microvascular complications and glycemic control in type 2 diabetes mellitus – A study at a tertiary care center in Delhi. *Hematol Transfus Cell Ther.,* **41**(1): 31-36.

Abouammoh NA and Alshamrani MA (2020). Knowledge about diabetes and glycemic control among diabetic patients in Saudi Arabia. *J. Diabetes Res.,* **2020**: 1-6.

Antunes BM, Rossi FE, Oyama LM, Rosa-Neto JC and Lira FS (2020). Exercise intensity and physical fitness modulate lipoproteins profile during acute aerobic exercise session. *Sci. Rep.,* **10**(1): 1-8.

Chen R, Yan J, Liu P, Wang Z and Wang C (2017). Plasminogen activator inhibitor links obesity and thrombotic cerebrovascular diseases: The roles of PAI-1 and obesity on stroke. *Metab. Brain Dis,,* **32**(3): 667-673.

Dastgheib SA, Najafi F, Shajari A, Bahrami R, Asadian F, Sadeghizadeh-Yazdi J, Akbarian E, Emarati SA and Neamatzadeh H (2020). Association of plasminogen activator inhibitor-1 4G5G Polymorphism with risk of diabetic nephropathy and retinopathy: A systematic review and meta-analysis. *JDMD***,** pp.1-12.

El Wakeel MA, Abou-El-Asrar Mohamed, El-Kassas, GM, Elabd MA, Zeid DA, Sabry RN and Awadallah EMAN (2017). Urinary markers of oxidative DNA damage in Type 1 diabetic children: Relation to microvascular complications. *Asian J. Pharm. Clin. Res.*, **10**(10): 318-22.

El Sayed ZH, Ismail SM and ELhakeem HA (2018). Evaluation of plasma fibrinogen and plasminogen activator inhibitor-1 in type 2 diabetes mellitus patients with coronary artery disease. *SSAMFG*, **2**(3): 252.

Grandl G and Wolfrum C (2018). February. Hemostasis, endothelial stress, inflammation, and the metabolic syndrome. *Semin Immunopathol.,* **40**(2): 215-224.

Jung RG, Simard T, Labinaz A, Ramirez FD, Di Santo P, Motazedian P, Rochman R, Gaudet C, Faraz MA, Beanlands RS and Hibbert B (2018). Role of plasminogen activator inhibitor-1 in coronary pathophysiology. *Thromb. Res,,* **164**: 54-62.

Kayapinar O, Ozde C and Kaya A (2019). Relationship between the reciprocal change in inflammation-related biomarkers (fibrinogen-to-albumin and hsCRP-to-albumin ratios) and the presence and severity of coronary slow flow. *Clin. Appl. Thromb. Hem.,* **25**: 1076029619835383.

Lee SJ, Hong JM, Lee SE, Kang DR, Ovbiagele B, Demchuk AM and Lee JS (2017). Association of fibrinogen level with early neurological deterioration among acute ischemic stroke patients with diabetes. *BMC Neurology*, **17**(1): 1-7.

Liu S, Li Y, Fan X, Li K, Xu C, Zhang L, Luo M, Wang L, Li R and Wu J (2020). Transplantation of adipose tissue lacking PAI-1 improves glucose tolerance and attenuates cardiac metabolic abnormalities in high-fat diet-induced obesity. *Adipocyte*, **9**(1): 170-178.

Persil-Ozkan O, Yigit E and Yigit Z (2019). Does weight loss affect the parameters that are metabolically related to cardiovascular diseases? *Saudi Med. J,,* **40**(4): 347.

Razak MKA and Sultan AA (2019). The importance of measurement of plasma fibrinogen level among patients with type-2 diabetes mellitus. *Diabetes Metab Syndr Clinical Research & Reviews,* **13**(2): 1151-1158.

Recinella L, Orlando G, Ferrante C, Chiavaroli A, Brunetti L and Leone S (2020). Adipokines: New potential therapeutic target for obesity and metabolic, rheumatic, and cardiovascular diseases. *Front. Physiol.,* **11**: pp.1-32.

Song C, Burgess S, Eicher JD, CHARGE Consortium Hemostatic Factor Working Group, ICBP Consortium, CHARGE Consortium Subclinical Working Group, O'Donnell CJ, Johnson AD, CHARGE Consortium Hemostatic Factor Working Group, Huang J and Sabater-Lleal M (2017). Causal effect of plasminogen activator inhibitor type 1 on coronary heart disease. *J. Am. Heart Assoc.,* **6**(6): e004918.

Mohiuddin SS 2018. Correlation of glycemic status with plasma fibrinogen level in insulin dependent as well as noninsulin dependent diabetic patients. *J. Endocrinol. Diab.*, **5**(5): 1-6.

Somodi S, Seres I, Lorincz H, Harangi M, Fulop P and Paragh G (2018). Plasminogen activator inhibitor-1 level correlates with lipoprotein subfractions in obese nondiabetic subjects. *Int. J. Endocrinol.,* **2018**(2-3): 1-9.

Sobolewski K, Radparvar S, Wong C and Johnston J (2018). Blood, blood components, plasma, and plasma products. *Side Eff. Drugs Annu.,* **40**: 415-429.

Shemran KA (2020). Study the elevation of fibrinogen level in plasma patients with diabetes mellitus type 2 in babylon governorate. *Indian J. Forensic Med. Toxicol.,* **14**(4): 2051.

Tabakcı MM, Gerin F, Sunbul M, Toprak C, Durmus HI, Demir S, Arslantas U, Cersit S, Batgerel U and Kargın R (2017). Relation of plasma fibrinogen level with the presence, severity, and complexity of coronary artery disease. *Clin. Appl. Thromb. Hem.,* **23**(6): 638-644.

Vousden KA, Lundqvist T, Popovic B, Naiman B, Carruthers AM, Newton P, Johnson DJ, Pomowski A, Wilkinson T, Dufner P and de Mendez I (2019). Discovery and characterisation of an antibody that selectively modulates the inhibitory activity of plasminogen activator inhibitor-1. *Sci. Rep.,* **9**(1): 1-12.

Zhang J, Wang Y, Zhang R, Li H, Han Q, Wu Y, Wang S, Guo R, Wang T, Li L and Liu F (2018). Serum fibrinogen predicts diabetic ESRD in patients with type 2 diabetes mellitus. *Diabetes Res. Clin. Pract.,* **141**: 1-9.

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