Original Research Article

TITLE: Relationship among HbA1c and some Markers of Endothelial Damage in type 2 Diabetes Mellitus

ABSTRACT

Background: A number of processes regulating the thrombolytic balance are impaired in diabetic patients as a result of dysfunction of endothelial cells leading to a hypercoagulative state. Von Willebrand factor (VWF) is an important marker of endothelial dysfunction. Plasminogen activator inhibitor-1 antigen (PAI-1-Ag), the major physiological inhibitor of tissue plasminogen activator (tPA), is mainly produced by endothelium. The aim of this study is to measure plasma levels of von Willebrand factor, Plasminogen activator inhibitor-1 antigen in type 2 diabetes mellitus patients and to correlate with glycated haemoglobin (HbA1c).

Study design: This prospective cohort study was conducted on 30 diagnosed type 2 DM patients who were about to start treatment.

Place and Duration of Study: Medical outpatient (MOP) clinic of Enugu State University of Science and Technology Teaching Hospital (ESUTTH), between January and December 2016.

Methodology: We included 30 patients (13 men, 17 women; age range 40-80 years) with type 2 diabetes mellitus. Blood samples were drawn from the patients before they commenced treatment, six months into the treatment and at twelve months of the treatment. Blood samples were also drawn from 25 age matched non diabetic patients. Plasma von Willebrand factor and Plasminogen activator inhibitor-1 antigen levels were determined by Enzyme linked immunosorbent assay. Glycated haemoglobin (HbA1c) and fasting blood sugar (FBS) levels were also evaluated along with them.

Results: This study was conducted on 30 type 2 DM patients consisting of 13 males and 17 females. At treatment naïve, mean levels of vWF were significantly increased (*45.48 +/- 6.46*) in male type 2 Diabetic patients compared to the control (*20.45 +/- 0.26*). Six months into treatment mean levels of vWF were significantly increased (*48.18 +/- 4.99*) in female type 2 Diabetic patients compared to the control (*37.64 +/- 7.93*). The plasma levels of vWF were significantly and positively correlated with HbA1c at six months into treatment in male type 2 DM patients. The plasma levels of vWF were also significantly and positively correlated with PAI-1 at six and twelve months into treatment in both genders.

Conclusion: There was strong significant positive correlation between plasma levels of vWF and PAI-1 in type 2 diabetes mellitus patients.

Keywords: endothelial, glycated, haemoglobin, type 2 diabetes, plasminogen activator, von Willebrand factor

1. INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder with diverse aetiologies which is known by chronic hyperglycaemia and the development of chronic vascular complications [1]. The International Diabetes Federation (IDF) estimated that, in 2011, 366 million people worldwide were already living with diabetes and that 80% of these individuals were based in low and middle income countries. It is expected that, by 2030, the number would have increased to 552 million. T2DM is widely known as a major public health problem, and this result in more than 90% of all diabetes cases. The indirect and initially asymptomatic nature of the disease leads to patients not seeking timely medical attention, so that 30-85% of cases of type 2 diabetes mellitus stay undiagnosed. At the time the diagnosis will be finally made, almost 20% of patients will be discovered to have complications of the disease [1]. Endothelial dysfunction is recognized to occur in type 2 Diabetes Mellitus (DM), which plays an important role in the development of atherosclerosis, a process that occurs prematurely and at an alarming rate in diabetic patients [2,3] Vascular endothelium damage is noted by increase in the plasma levels of endothelial markers like von Willebrand factor (vWF), plasminogen activator inhibitor-1 (PAI-1), selectin and thrombomodulin. These markers may be helpful to investigate early endothelium involvement. Plasminogen activator inhibitor-1 (PAI-1) also known as endothelial plasminogen activator inhibitor or serpin E1 is a protein that in humans is encoded by the *SERPINE1* gene. The PAI-1 gene is *SERPINE1*, located on chromosome 7 (7q21.3-q22). Increase in plasma PAI-1 appears to jeopardize normal fibrin clearance mechanisms and encourage thrombosis. The plasminogen activators (tissue plasminogen activator and the urokinase type activator) convert plasminogen to plasmin, which is required in fibrinolysis, tissue rearrangement and cell migration [4]. In large epidemiological studies high plasma PAI-1 has been shown in various subgroups as an essential feature of T2D [5,6,7] and this increase may lead to a thrombotic tendency [7]. An increased level of von Willebrand factor (vWF) shows activation and damage to endothelial cells and has been illustrated in association with atherosclerosis and diabetes. High concentration levels of von Willebrand factor (vWF) has also been shown in certain inflammatory or atherosclerotic vascular incidents and signify a degeneration of endothelial cells [8,9]. Von Willebrand factor (vWF) has shown to be a predictive marker of diabetic nephropathy, which indicates that endothelial dysfunction facilitates the onset of diabetic microangiopathy. Based on our findings the plasma levels of plasminogen activator inhibitor 1 antigen and Von Willebrand factor (vWF) antigen levels in type 2 diabetes mellitus has not been established in people residing in Enugu. Therefore, the aim of this study was to measure the plasma levels of PAI-1, vWF in type 2 diabetes mellitus patients and to correlate them with glycated haemoglobin.

2. MATERIALS AND METHOD

This prospective cohort study involved diabetic patients that were referred to medical outpatient clinic between January and March, 2016. The patients were screened for eligibility. The study was approved by the Research Ethics Committee of Enugu State University of Science and Technology Teaching Hospital (ESUTH) Enugu. Written informed consent was obtained from each subject. A total number of 50 participants were recruited for this study. The study population is 30 known type 2 diabetic patients comprising of 13 male and 17 female aged 40-80years were investigated. Twenty five age-matched non diabetic individuals who had no previous history of type 2 diabetes mellitus were included as control subjects. The mean age for the patients was 67.2±9.5 for the male and 60.7±12.5 for female. Inclusion criteria were individuals exceeding 40 years of age, about to commence treatment. Those who were unable to sign inform consent form, could not observe a minimum of 10-12 hours fast, and who had other chronic diseases were excluded. Subjects were divided into two groups based on their gender. Blood samples for plasma plasminogen activator inhibitor-1 antigen, von Willebrand factor antigen, glycated hemoglobin and fasting blood sugar were drawn from each patient before treatment, 6 months into treatment and 12 months of treatment.

2.1 HAEMATOLOGICAL ANALYSES

Plasma plasminogen activator inhibitor-1 antigen and von Willebrand factor antigen were measured by AssayMax ™ ELISA Kits from Assaypro (3400 Harry S Truman Blvd St.Charles, MO 63301 USA). Glycated hemoglobin was determined by glycohaemoglobin reagent set from Teco diagnostic (Lakeview Avenue Anaheim, California, U.S.A).

2.1.1 STATISTICAL ANALYSIS

The results were analyzed by the SPSS 21.0 (Statistical Package of Social Science). The data were expressed as mean ± SD. The group comparisons were determined by ANOVA. The association between PAI-1, vWF and glycated haemoglobin was determined by Pearson correlation. P value less than 0.05 was considered significant.

3. RESULT

Table 1 showed mean±SD of PAI-1, vWF, HbA1c and FBS of male patients at treatment naïve, 6 months of treatment, 12 months of treatment and control subjects. The vWF of male control subjects (20.45+/-0.26 mU/ml) were significantly lower compare with treatment naive (45.48+/-6.46 mU/ml), 6 months treatment (42.30+/-5.98 mU/ml) and 12 months of treatment (44.80 +/- 2.22 mU/ml) respectively (F = 63.30; *p = .00*). The HbA1c of male subjects at treatment naïve (9.45+/-1.59 %) were significantly higher compare with 6 months treatment (5.76+/-0.49 %), 12 months treatment (6.96+/-0.06 %) and control (5.40+/-1.79 %) respectively (F=29.88; *p = .00*). Table 2 showed mean±SD of PAI-1, vWF, HbA1c and FBS of female patients at treatment naïve, 6 months of treatment, 12 months of treatment and control subjects. The vWF of female control subjects (37.64+/-7.93 mU/ml) were significantly lower compare with vWF level at 6 months treatment (48.18+/-4.99 mU/ml). The HbA1c of female subjects at treatment naïve (9.08+/-1.64 %) were significantly higher compare with 6 months treatment (6.95+/-1.17 %), 12 months treatment (6.83+/-0.16 %) and control (5.15+/-2.08 %) respectively (F= 19.19; *p = .00*). Table 3 showed Correlation of haematological parameters with glycated haemoglobin in male and female patients. At six months, vWF showed moderate positive significant correlation with HbA1c in male individuals (r= 0.572; *p = .041*). PAI-1 showed strong negative correlation with vWF at six months (r=-0.771; *p = 0.002*) and strong positive correlation at twelve months (r =1.000; *p =.000*) in male patients. PAI-1 showed strong positive correlation with vWF both at six months (r=-0.638; *p = 0.006*) and twelve months (r =0.722; *p =.001*) in female patients.

Table 1 Haematological parameters of the male subjects

	PAI-1 (ng/ml)	vWF (mU/ml)	HbA1c (%)	FBS (mmol/L)
T_N	3.67±0.35	45.48±6.46	9.45±1.59	7.77±3.27
T_6	3.56±0.37	42.30±5.98	5.76±0.49	6.05±0.57
T_{12}	3.52±0.03	44.80±2.22	6.96±0.06	5.36±1.13
C	3.69±0.33	20.45±0.26	5.40±1.79	5.24±0.35
F (p) value	0.98(0.41)	63.30(0.00)	29.88(0.00)	5.10(0.00)

T_N vs T_6 (p) value	0.92	0.64	0.00	0.29
T_N vs T_{12} (p) value	0.24	0.99	0.00	0.10
T_N vs C (p) value	1.00	0.00	0.00	0.07
T_6 vs T_{12} (p) value	0.85	0.66	0.00	0.24
T_6 vs C (p) value	0.87	0.00	0.92	0.00
T_{12} vs C (p) value	0.22	0.00	0.09	0.99

Key = $p < .05$

Abbreviation: T_N=treatment naïve, T_6= 6 months into treatment, T_{12}= 12 months into treatment, c=control

Table 2 Haematological parameters of the female subjects

	PAI-1 (ng/ml)	vWF (mU/ml)	HbA1c (%)	FBS (mmol/L)
T_N	3.74±0.34	43.41±6.92	9.08±1.64	8.60±2.49
T_6	3.57±0.41	48.18±4.99	6.95±1.17	7.71±2.58
T_{12}	3.79±0.25	45.08±4.16	6.83±0.16	7.11±0.35
C	3.66±0.59	37.64±7.93	5.15±2.08	5.00±0.71
F (p) value	0.90(0.45)	6.33(0.00)	19.19(0.00)	7.65(0.00)
T_N vs T_6 (p) value	0.58	0.13	0.00	0.73
T_N vs T_{12} (p) value	0.97	0.84	0.00	0.10
T_N vs C (p) value	0.98	0.26	0.00	0.00
T_6 vs T_{12} (p) value	0.31	0.27	0.98	0.77
T_6 vs C (p) value	0.97	0.01	0.11	0.00
T_{12} vs C (p) value	0.91	0.07	0.12	0.00

Key = $p < .05$

Table 3 Correlation of haematological parameters with glycated haemoglobin in both sexes

	Treatment naïve		six months		twelve months	
Male variables	(r)	p-value	(r)	p-value	(r)	p-value
PAI-1 vs HbA1c	-0.391	0.186	-0.355	0.235	0.127	0.679
vWF vs HbA1c	0.006	0.985	0.572	0.041	-0.098	0.751
PAI-1 vs vWF	0.552	0.050	-0.771	0.002	1.000	0.000
Female variables						
PAI-1 vs HbA1c	-0.238	0.358	-0.114	0.663	0.099	0.705
vWF vs HbA1c	-0.299	0.244	-0.083	0.752	0.377	0.136
PAI-1 vs vWF	0.054	0.837	0.638	0.006	0.722	0.001

4. DISCUSSION

A number of prospective studies have shown that circulating biomarkers of endothelial dysfunction such as von Willebrand factor forecast incident diabetes [10, 11, 12]. Circulating levels of t-PA and vWF are associated with risk of coronary heart disease, and increase levels in individuals with type 2 diabetes might therefore promote their atherothrombotic complications [13]. In this study, an increased in plasma vWF level in male type 2 diabetics in comparison with the control subjects was found. This observed increase vWF levels in type 2 diabetic patients in comparison with the control subjects were also seen in the work of Cihangir *et al.*, 2005; Umadevi *et al*., 2016, they observed that plasma level of vWF were significantly increased in the type 2 diabetic patients compared with the healthy subjects [14,15]. Persons with higher plasma levels of PAI-1 and von Willebrand factor (vWF) are at an increased risk of, acute myocardial infarction, pulmonary embolism and diseases of peripheral blood vessels [16]. In this study vWF was positively correlated with HbA1c at six months. This finding contrast with study carried on Type 2 Diabetic Patients with and without Diabetic Vascular Complications, in which there was no significant positive or negative correlation between vWF and HbA1c [14]. PAI-1 and vWF may be used as biomarkers for the diagnosis of endothelial cell dysfunction. Endothelial cell dysfunction in someone with metabolic syndrome may be connected with obesity, blood sugar and blood fat [17]. In this study PAI-1 was positively correlated with vWF in both male and female type 2 diabetes mellitus. This finding contrast with study done by Cihangir *et al*., 2005, in which vWF activity was negatively correlated with plasma t-PA levels [14]. Study had shown that most of circulating t-PA antigen is coupled in complex with PAI-1. And also Close correlation between t-PA and PAI-1 can be shown by the increased level of t-PA/PAI-1 complexes because the PAI-1 level is essential for t-PA level [18].

CONCLUSION

Plasma von Willebrand factor antigen levels was found to be significantly higher in type 2 diabetes mellitus and also positively correlated with Plasminogen activator inhibitor-1 antigen in both male and female type 2 diabetes mellitus patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist

LIMITATIONS OF THE STUDY

There is no data on blood pressure, BMI and waist circumference of the patients. Small sample size was also used in this study.

REFERENCES

1. Amod A, Ascott-Evans BH, Berg GI, Blom DJ, Brown SL, Carrihill MM. SEMDSA Guideline for the management of Type 2 Diabetes (Revised). Diabetes. 2012;17(2):1-95.

2. Kolluru GK, Bir SC, Kevil CG. Endothelial dysfunction and diabetes: effects on angiogenesis, vascular remodelling, and wound healing. International Journal *of* Vascular Medicine. 2012;2012:1-30.

3. Avogaro A, Albiero M, Menegazzo L, de Kreutzenberg S, Fadini GP. Endothelial Dysfunction in Diabetes. Diabetes Care. 2011;34(Supplement 2):S285–S290.

4. Syrovets T, Lunov O, Simmet T. Plasmin as a proinflammatory cell activator. Journal of Leukocyte Biology. 2012;92:509–519.

5. Festa A, D'Agostino R, Tracy RP, Haffner SM. Insulin Resistance Atherosclerosis Study. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: The Insulin Resistance Atherosclerosis Study. Diabetes. 2002; 51:1131–7.

6. Gavriilaki E, Gkaliagkousi E, Nikolaidou B, Triantafyllou G, Chatzopoulou F, Douma S. Increased thrombotic and impaired fibrinolytic response to acute exercise in patients with essential hypertension: the effect of treatment with an angiotensin II receptor blocker. Journal of Human Hypertension. 2014;28:606–609.

7. Eliasson MC, Jansson JH, Lindahl B, Stegmayr B. High levels of tissue plasminogen activator (tPA) antigen precede the development of type 2 diabetes in a longitudinal population study. The Northern Sweden MONICA Study. Cardiovascular Diabetology. 2003;2:19.

8. Murthy SK, Nguyen GC. Venous thromboembolism in inflammatory bowel disease: an epidemiological review. American *Journal of* Gastroenterology. 2011;106:713–718.

9. Wannamethee SG, Sattar N, Rumley A, Whincup PH, Lennon L, Lowe GD. Tissue plasminogen activator, von willebrand factor, and risk of type 2 diabetes in older men. Diabetes Care 2008;31(5):995-1000

10. Festa A, Williams K, Tracy RP, Wagenknecht LE, Haffner SM. Progression of plasminogen activator inhibitor-1 and fibrinogen levels in relation to incident type 2 diabetes. Circulation. 2006;113:1753–1759.

11. Meigs JB, Wilson PW, Fox CS, Vasan RS, Nathan DM, Sullivan LM, D'Agostino RB. Body mass index, metabolic syndrome, and risk of type 2 diabetes or cardiovascular disease. Journal of Clinical Endocrinology Metabolism. 2006;91:2906–2912.

12. Thorand B, Baumert J, Chambless L, Meisinger C, Kolb H, Doring A, Lowel H, Koenig W. Elevated markers of endothelial dysfunction predict type 2 diabetes mellitus in middle-aged men and women from the general population. Arteriosclerosis, Thrombosis, and Vascular Biology.2006;26:398–405.

13. Wannamethee SG, Lowe GDO, Shaper AG, Rumley A, Lennon L, Whincup PH. Insulin resistance, haemostatic and inflammatory markers and coronary heart disease risk factors in type 2 diabetes with and without coronary heart disease. Diabetologia. 2004;47:1557–1565.

14. Cihangir E, Arif H, Sükrü Ç, Ercüment O, ÖnderErsöz H, Kubilay U, Orhan D, Münir T. Coagulation and Fibrinolysis Parameters inType 2 Diabetic Patients with and without. Diabetic Vascular Complications Medical Principles Practice. 2005;14:22–30.

15. Umadevi B, Roopakala M. S, Wilma Delphine Silvia C. R, Prasanna Kumar K. M. Role of von willebrand factor in type 2 diabetes mellitus patients. Journal of Evolution of Medical and Dental Sciences. 2016;5(81):6075-6079.

16. Gorog D. A. Prognostic value of plasma fibrinolysis activation markers in cardiovascular disease. Journal of American College of Cardiology. 2010;55:2701–2709.

17. Alessi MC, Juhan-Vague I. PAI-1 and the metabolic syndrome: links, causes, and consequences. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006;26:2200–2207.

18. Al-Hamodi Z, Ismail I.S, Saif-Ali R, Ahmed KA. Muniandy S. Association of plasminogen activator inhibitor-1 and tissue plasminogen activator with type 2 diabetes and metabolic syndrome in Malaysian subjects. Cardiovascular *Diabetology*. 2011;10: 23.