Tissue factor levels in Type 2 Diabetes Mellitus

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Abstract

Introduction: Type 2 diabetes mellitus is a pandemic associated with disturbance in haemostasis that could contribute to the development of diabetic vascular disease and accelerated atherosclerosis. In this population, hypercoagulation is prevalent, as well as pathological changes to erythrocytes. This is mainly due to upregulated circulating inflammatory markers.

Materials and methods: Here we looked at tissue factor (TF) levels using ELISA, in a sample of diabetics, with and without cardiovascular complications. Diabetic subjects were recruited from the diabetic clinic at Steve Biko Academic Hospital, Pretoria, South Africa. 20 diabetics with cardiovascular disease and 22 without were enrolled to participate.

Results and conclusion: TF levels were significantly elevated in both diabetic groups when compared to the controls. We suggest that pathologic plasma TF activity, as marker of increased propensity of clot pathology, should be investigated. Agents that might lower TF levels might also possibly lower thrombotic complications.

INTRODUCTION

Type 2 diabetes (which is almost always associated with cardiovascular involvement), shows an uncontrollable upward trend, as seen in the latest data published by the WHO (*Global Report on Diabetes:* http://www.who.int/diabetes/global-report). Individuals with chronically elevated glucose and/or insulin levels, as is present in most patients with type 2 diabetes, have accelerated atherosclerosis and are also prone to acute vascular events (Vaidyula et al., 2006). Type 2 diabetes is also associated with disturbances in haemostasis that could contribute to the development of diabetic vascular disease (Morishita et al., 1996, Ferreiro et al., 2010) (Alzahrani and Ajjan, 2010). These pandemic increases in numbers with cardiovascular co-morbidities, including atherothrombotic events and higher incidence of coronary artery disease, are mainly because of the increasing obesity tendency. All of the mentioned co-morbidities are frequently associated with a worse prognosis in type 2 diabetes patients, compared with non-diabetic patients (Virmani and Roberts, 1983).

The classical view of the main role of tissue factor (TF) as the initiator of the coagulation cascade has to be reassessed, as it is also a mediator in the pathogenesis of cardiovascular disorders (Breitenstein et al., 2010, Chu, 2011). Atherosclerosis contributory risk factors such as smoking, hypertension, hyperlipidaemia and diabetes all increase TF expression (Tatsumi and Mackman, 2015, Bode and Mackman, 2015). The discovery of blood-borne TF has changed the opinion of labelling vessel-wall TF as the major determinant of thrombosis as emerging studies have recognised a hypercoagulable state associated with an increased circulating TF activity with the development of the concept of ‘vulnerable

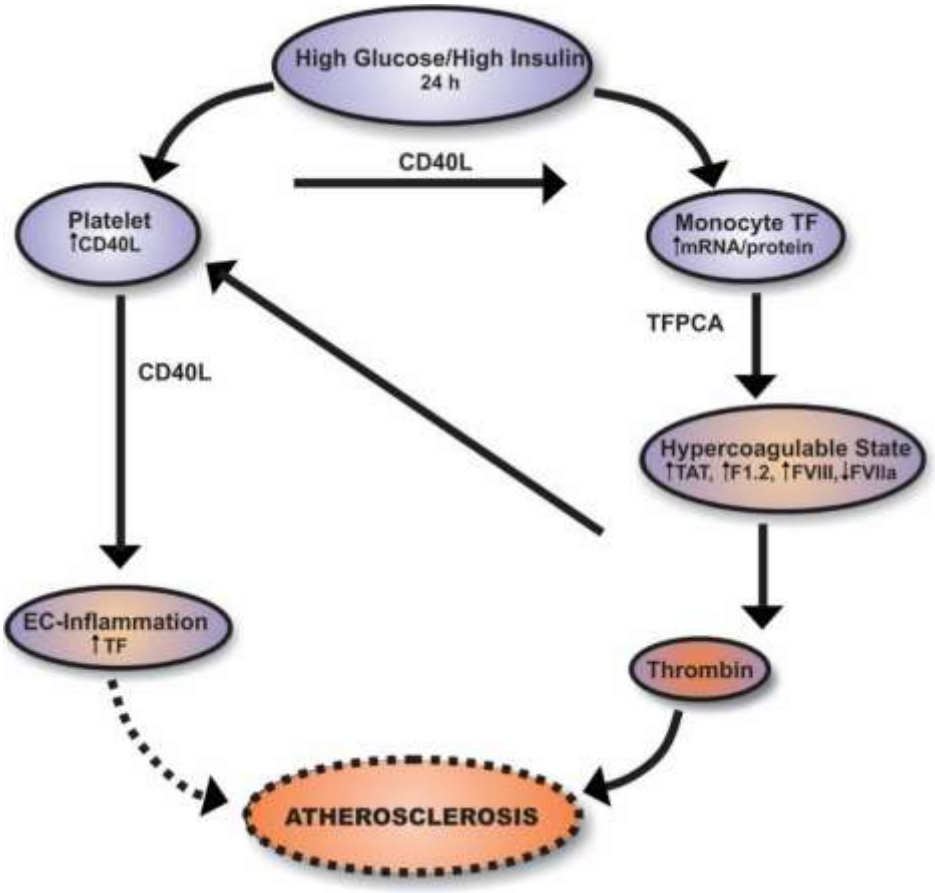
blood' (Cimmino et al., 2011). In addition, it has been shown that this circulating pool of TF in blood that is associated with cells and microparticles is thrombogenic (Rauch and Nemerson, 2000) is elevated in type 2 diabetes mellitus (Sambola et al., 2003).

Under physiological conditions, the production/activation of prothrombotic and fibrinolytic factors are finely tuned and well balanced, so that haemostasis is appropriate, sufficient to protect from bleeding yet adequately suppressed to prevent pathological thrombosis. However, in diabetes, this fine balance is disturbed and tipped towards a prothrombotic/hypofibrinolytic phenotype which, in association with atheromatous vascular changes and platelet hyperactivity, poses an increased predisposition to cardiovascular ischaemic events (Alzahrani and Ajjan, 2010).

Multiple mechanisms have been implicated, but particularly pathological changes of the coagulation and resulting abnormal fibrinolysis, feature prominently in type 2 diabetes (Balasubramaniam et al., 2012). The study by Vaidyula and co-workers, tested the hypothesis that hyperglycaemia and/or hyperinsulinaemia singly or combined may increase TF, and highlighted the following results: (a) in healthy volunteers, combined elevation of plasma insulin and glucose levels for 24 hours produced (b) a nine fold increase in circulating tissue factor procoagulant activity (TFPCA) associated with an increase in monocyte TF surface expression and mRNA and (c) changes in other components of blood coagulation. See Figure 1 (Vaidyula et al., 2006). Despite treatment with the recommended dual antiplatelet therapy to treat these coagulation pathologies is, there is still an increased atherothrombotic risk in these patients. This may partly be due to abnormal endothelial function, abnormal platelet haemostasis resulting in platelet hyperactivity and/or a general

dysregulation of the coagulation process (Storey, 2010). It is thus evident that more effective therapies and novel markers as well as additional disease tracking methods, are warranted for this group of high risk patients. In this paper, we look at TF levels of two groups of type 2 diabetic patients, those with and those without cardiovascular complications.

FIGURE 1: Proposed scheme mechanisms explain changes in TF procoagulant activity, factor VII, factor VIII, thrombin-antithrombin (TAT), prothrombin fragment (F1.2) and CD40 ligand (CD40L) in normal subjects in response to hyperglycaemia. Taken from Vaidyula et al. (Vaidyula et al., 2006) TFPCA = tissue factor procoagulant activity, EC = endothelial cell.



MATERIALS AND METHODS

Participants

42 diabetic subjects were recruited from the Steve Biko Academic Hospital, diabetic clinic in South Africa. Ethical clearance was obtained for this study from the University of Pretoria Human Ethics Committee. Informed consent was obtained from all participants. Inclusion criteria included: (a) subjects older than 18 years and willing to provide informed consent, (b) subjects with known diagnosis of diabetes, (c) for the cardiovascular group, history of previous myocardial infarction, peripheral arterial disease, stroke or coronary arterial bypass grafting. Exclusion criteria included: (a) subjects hemodynamically unstable and (b) subjected with documented life threatening disease (malignancy, HIV/AIDS). Two groups were distinguished, 20 diabetics with cardiovascular disease (CVD) and 22 without CVD.

TF Assay Procedure

The ELISA assay (IMUBIND tissue factor ELISA kit no. 845, American Diagnostica Inc., Stamford) was used for the quantitative determination of human TF, in plasma at an absorbance at 450 nm and comparing the values with those of a standard curve. Final measurements were calculated by deducting the background average of the blanks from the standards and sample readings. A standard curve was drawn by plotting the mean absorbance for each TF standard against the corresponding concentration of TF. The level of TF was expressed in $pg.mL^{-1}$.

Statistical analysis

All statistical analyses were performed using the GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. Mann-Whitney test was used to compare the two diabetic groups, with a $P \leq .05$ considered significant.

RESULTS

Characteristics of the study population are shown in Table 1. Table 1 provides information on the controls and two diabetic groups. The CVD group were slightly older than the group without CVD, mean age of 61 years and 53 years respectively. It is notable to observe that both groups had uncontrolled glucose as evidenced by HBA1c values. Detail of the chronic medication taken by patients are also shown. Our results in Table 2, indicate that the concentration of TF was increased in both diabetic groups (with and without CVD) when compared to normal values, however there was no significant difference between diabetics without CVD and diabetics with CVD. However, the TF values in both diabetic groups were almost two and a half times greater than the control value.

TABLE 1: Baseline demographic data and clinical characteristics of the study population.

Variable	Diabetics without CVD (n=22)	Diabetics with CVD (n=20)
Age, years	60 ± 8.7	52 ± 14.9
Males, n (%)	8 (36)	13 (65)
Females, n (%)	14 (63)	7 (33)
Diabetic treatment:		
Insulin, n (%)	4 (18)	3 (15)
Oral agents only, n (%)	18 (82)	16 (80)
Oral and insulin, n (%)	7 (32)	11 (55)
HBA1c[a] %	9.0 ± 2.6	8.5 ± 1.7
Cardiovascular Complications		
Previous MI[b], n (%)	n/a	9 (45)
PAD[c], n (%)	n/a	2 (10)
CABG[d], n (%)	n/a	9 (45)
Essential medication		
ACEI[e], n (%)	7 (32)	12 (60)
Ca-antagonist[f], n (%)	4 (18)	1 (5)
B-blocker, n (%)	3 (14)	4 (20)
Nitrates, n (%)	1 (5)	11 (55)
Statins, n (%)	9 (41)	17 (85)
Disprin, n (%)	6 (27)	13 (65)
Warfarin, n (%)	1 (5)	3 (15)

Data expressed as mean ± (SD) or n (%)

[a]HBA1c = haemoglobin A1c (not all 42 subjects had this test completed and are available for 50% of the subjects).
[b]MI = Myocardial infarction
[c]PAD = Peripheral arterial disease
[d]CABG = coronary arterial bypass grafting
[e]ACEI = Angiotensin converting enzyme inhibitor
[f]Ca-antagonist = Calcium antagonist
n/a = not applicable

TABLE 2: TF levels and for type 2 diabetic patients with and without CVD.

	Diabetics without CVD (n=22)	Diabetics with CVD (n=20)	*P* value
Tissue factor [a]164.28 [a](117.39/183.85)	452.60 ± 163.80	428.50 ± 120.60	<0.0001

All values are shown as medians with standard deviation.

[a]Control value as recently calculated in study done by Ruszkowska-Ciastek et al (Ruszkowska-Ciastek et al., 2015) with lower/upper quartile in parenthesis.

DISCUSSION

According to the International Diabetic Federation, the number of people with diabetes worldwide is increasing and by 2030 this will have risen to 552 million (Whiting et al., 2011). Individuals with chronically elevated glucose and/or insulin levels, as is present in most patients with type 2 diabetes, have accelerated atherosclerosis and are prone to acute vascular events (Vaidyula et al., 2006). Evidence shows that diabetes has been considered to have a prothrombotic status. Characteristic findings in type 2 diabetes includes: increased coagulation, impaired fibrinolysis, endothelial dysfunction and platelet hyper-reactivity (Creager et al., 2003). Increased coagulation can be ascribed to the increased concentration of TF, FVIII, thrombin and fibrinogen (Alzahrani and Ajjan, 2010) (Ferreiro et al., 2010). Other mechanisms implicated for the prothrombotic milieu in diabetes is that of increased platelet activity (which increase thrombin expression (Martin-Timon et al., 2014). Diabetic subjects are also known to have elevated levels of plasminogen activator inhibitor type I (PAI-1) which contributes to the hypofibrinolysis (Olexa and Olexova, 2003, Fujii et al., 1998). Further evidence provided by Boden and co-workers, showed that high levels of TF in poorly controlled type 2 diabetics which feature hyperglycaemia and hyperinsulinaemia, contributes to the underlying low grade inflammation (Boden et al., 2007). Due to the low grade inflammation, there is a rise in circulating levels of interleukin-6 (IL-6), fibrinogen and TF expression in vascular cells (Martin-Timon et al., 2014). In an environment of chronic

hyperglycaemia, typical of type 2 diabetes, the binding of advanced glycated end products to their specific receptors creates an intravascular oxidative stress response, culminating in TF expression *in vitro* (Bierhaus et al., 1997). Furthermore, TF was discovered to be an independent factor related to microvascular diabetic complications (microalbuminuria, retinopathy and neuropathy) which is suggestive of endothelial dysfunction rather than procoagulant activity (Sommeijer et al., 2006). In diabetic subjects, particularly those with nephropathy, elevated tissue factor pathway inhibitor (TFPI) activity has been documented (Leurs et al., 1997). Physiologically, TFPI is the key factor in the initial phase of the coagulation pathway mediated by TF, controlling in turn, the production of thrombin which is so crucial in the pathophysiology of atherothrombosis (Opstad et al., 2010). More importantly, altered TF/TFPI ratio has been associated with the development of atherosclerosis, acute coronary syndrome, disseminated intravascular coagulation, sepsis or thrombotic complications related to malignancies (Ardissino et al., 1997) (Creasey and Reinhart, 2001).

The finding in our study of elevated TF levels in both diabetic groups, compares favourably with other studies where high TF levels are found in type 2 diabetic subjects (El-Hagracy et al., 2010, Ruszkowska-Ciastek et al., 2015, Boden et al., 2007). We additionally show that cardiovascular complications in diabetes further increase TF levels. Therefore the more “inflammatory” the individual, the higher the TF levels. Of note is the high incidence of dyslipidaemia and hypertensives among our participants, especially higher in the diabetic group with CVD. 85% of subjects in the CVD group were on statins compared to the 41% in the group without CVD. More subjects were on anti-hypertensive agents in the CVD group. There is an

increased tendency for thrombosis in the presence of dyslipidaemia as cholesterol parameters alters TF and TFPI expression in atheromatous plaque (Zawadzki et al., 2007). In the study by El-Hagracy, the diabetic dyslipidaemic and hypertensive diabetic patients also had significantly higher TF and TFPI plasma levels when compared to the non-dyslipidaemic patients (El-Hagracy et al., 2010). TF is therefore an important biomarker of the general inflammatory status of an individual, and lowering its levels might have significant implications for the health of particularly patients with type 2 diabetes.

CONCLUSION

Patients with diabetes presenting with acute coronary syndrome have a higher risk of cardiovascular complications and recurrent ischaemic events when compared to non-diabetic patients. Multiple mechanisms have been implicated amongst which abnormalities in coagulation and fibrinolysis feature prominently (Balasubramaniam et al., 2012). Despite being on currently recommended dual antiplatelet therapy, diabetes still poses an increased atherothrombotic risk (Storey, 2010). It is thus evident that more effective therapies are warranted for this group of high risk patients. It remains to be seen if perhaps pharmacological control of plasma TF activity, in particular pathologic TF expression or augmentation of TFPI will lower thrombotic complications.

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Conflict of interest disclosure

None to report.

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