

Blood viscosity at different stages of diabetes pathogenesis

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Introduction

Blood viscosity is one of Virchow's triad of subclinical risk factors for future cardiovascular disease (CVD).¹ Factors that increase whole blood viscosity (WBV) include haematocrit, total plasma protein, erythrocyte aggregation and erythrocyte deformability (ED).^{2,3} In the absence of the first two, increased viscosity can indicate decreased ED and fluidity and an intrinsic resistance to blood flow in the vascular system.³

Erythrocyte deformability is a physical property that enables the cells to change shape and flow smoothly with little or no aggregation/friction^{4,5} and is influenced by oxidative changes to the protein cytoskeleton of the erythrocyte membrane, oxidation of the haemoglobin molecule and/or peroxidation of the lipid membrane.^{6,7} Thus, erythrocyte oxidative stress (EOS) can make the blood more viscous, which can lead to the development of vascular abnormalities including atherothrombosis and endothelial dysfunction that are associated with coronary artery disease.^{8,9} These blood flow abnormalities can be detected as increased viscosity.

In a meta-analysis of prospective studies, thrombosis was considered to be reaching epidemic proportion partly due to type 2 diabetes mellitus (DM), with increased viscosity as an independent risk predictor.¹⁰ This suggests that blood viscosity is a biomarker of subclinical cardiovascular disease (SCVD) in diabetes. Whether or not whole blood viscosity varies with progression in diabetes, and EOS and blood viscosity are concomitantly mainly abnormal as yet is unknown. Such finding would be useful in defining the clinical utility of blood viscosity with regards to either specificity of a disease condition or sensitivity to a mechanistic cause of stasis.

Given that EOS makes the blood more viscous, it follows that abnormal EOS indices will be prevalent in abnormally high viscosity. To the authors' knowledge, this also has yet to be reported. The aim of the present study is to investigate any significant variation in blood viscosity at different stages

ABSTRACT

Hyperglycaemia-induced oxidative stress is implicated as a cause of increased whole blood viscosity (WBV), which is a clinically modifiable risk factor for cardiovascular disease (CVD). However, whether or not there is variation in WBV at different stages of diabetes mellitus (DM) has yet to be confirmed. The sensitivity of underlying oxidative stress has also yet to be investigated. A total of 154 participants representing different stages of DM pathogenesis were selected for the study. Healthy control, prediabetes, DM and DM+CVD groups were compared for variation in WBV levels. The prevalence of oxidative stress, indicated by abnormal levels of erythrocyte glutathione, malondialdehyde and methaemoglobin, associated with high WBV was evaluated. The results showed a statistically significant difference in WBV between groups ($P < 0.03$). The level of viscosity was significantly lower in the control group relative to the prediabetes group ($P < 0.01$) and DM+CVD group ($P < 0.04$). There was no statistically significant difference between the DM+CVD and prediabetes groups. Greater than 76% prevalence of oxidative stress was shown to be associated with high WBV, reaching 95% prevalence in prediabetes. The study showed that WBV varies between individuals with different stages of diabetic macrovascular pathogenesis, including prediabetes. Redefining the criteria for use of WBV on the basis of sensitivity to underlying oxidative stress, rather than specificity to a disease condition, means that this easily performed test is an option to consider in an all-inclusive laboratory approach to early intervention against future diabetic macrovascular complications. This is particularly important for individuals with subclinical hyperglycaemia.

KEY WORDS: Blood viscosity.
Cardiovascular diseases.
Diabetes mellitus.
Oxidative stress.

of diabetes, and whether or not there is prevalence of abnormal EOS indices in high blood viscosity, with or without hyperglycaemia. Furthermore, it seeks a rationale to consider viscosity as a biomarker of SCVD in diabetes pathology.

Materials and methods

This study utilised de-identified data from the evaluation of EOS in diabetes and its cardiovascular complications,¹¹ which was approved by the Ethics Committee of the Charles

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Sturt University. The re-use of de-identified data was in accordance with the university's policy. Specifically approved volunteers ($n=620$) participated in the study, of which 266 were selected and distributed into seven groups (control, family history of diabetes, prediabetes, prediabetes with CVD, DM, DM+CVD and CVD) based on clinical history/status. Definitions of the groups and selection criteria, as well as the baseline reference control values, were as previously published.¹¹

The data for this study were limited to samples collected between July 2004 and 2005. Among 162 participants that constituted the control ($n=49$), prediabetes ($n=41$), DM ($n=30$) and DM+CVD ($n=42$) groups for this study, eight had no results for viscosity on the same blood samples from which EOS data for this study were obtained, and these were excluded. Therefore, 154 participants were selected from the four groups and re-used for this study (Table 1).

The laboratory determination of EOS indices (erythrocyte glutathione [GSH], malondialdehyde [MDA] and methaemoglobin) was performed according to Richards *et al.*,^{12,13} based on the 5,5-dithiobis-2-nitrobenzoic acid (DTNB) reaction method for GSH, thiobarbituric acid reactive substances (TBARS) principle for MDA, and cyanmethaemoglobin method for methaemoglobin. Whole blood viscosity was measured at a low shear rate using a Silenus viscometer (Silenus Instruments, Melbourne, Australia) as previously described by Richards *et al.*¹⁴

Statistics

It has been determined in a multivariate analysis (MANOVA) that whole blood viscosity does not show statistical significance in the presence of other confounding factors.¹¹ In this study, ANOVA was employed for comparison of viscosity as a single variable. The 95th percentiles were compared and Student's *t*-test was also performed to determine statistical significance between pairs of groups using Microsoft Excel. The prevalence of positive EOS indices was determined by sorting data by descending viscosity, using previously reported control mean values as baseline (Table 1), and critically evaluating the percentage of those above the baseline with an abnormal EOS index.

Table 1. Prevalence of abnormal EOS indices determined by sorting data by descending level of viscosity, and critically evaluating the percentage of those above the baseline with an abnormal EOS index.

	Control	preDM	DM	DM+CVD	Baseline values [†]
<i>n</i>	48	37	29	40	
Gender (f/m)	29/19	15/22	11/18	22/18	
Mean age (years)	56	59	59	66	
Viscosity [‡]	21 (44%)	22 (59%)	12 (41%)	22 (55%)	81 mPas
GSH [‡]	57%	77%	67%	73%	67 mg/dL
MDA [‡]	19%	55%	33%	64%	29 nmol/mL
MetHb [‡]	24%	36%	42%	59%	0.75 %
Positive [§] EOS index	76%	95%	92%	91%	

[†]Based on average levels in our control group.¹¹

[‡]Whole blood viscosity at low shear rate.

[§]Abnormal level concomitant with abnormal viscosity.

[§]At least one EOS index abnormality concomitant with abnormal viscosity per participant.

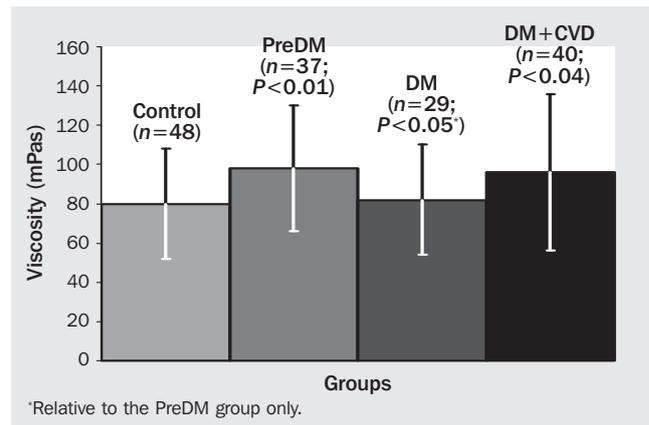


Fig. 1. Comparison of whole blood viscosity levels between groups (ANOVA: $P < 0.02$).

Results

A very high prevalence of abnormal levels of oxidative stress index was observed to be associated with high levels of WBV (Table 1). Comparison of groups by statistical analysis of variance (ANOVA) showed a significant difference between the groups ($P < 0.03$). Student's *t*-test analysis indicated a statistically significant lower WBV level in the control group relative to the preDM group ($P < 0.01$) and the DM+CVD group ($P < 0.04$). There was also a statistically significant difference between the preDM and the DM groups ($P < 0.05$, Fig. 1). Figure 1 also shows a lower mean blood viscosity, which is not statistically different between the DM+CVD and the DM groups.

Variation in WBV was shown to be associated with progression of diabetes pathology. Figure 1 shows that level of blood viscosity is statistically significantly lower in the DM group compared to the preDM group ($P < 0.05$). This observation may be due to the fact that members of the DM group were on hypoglycaemic and/or antiplatelet drug therapy. However, no statistically significant difference was seen in participants in the DM+CVD group, although members of the control group showed a significantly lower level ($P < 0.04$).

Discussion

This study suggests a progressively increasing level of WBV in all stages of hyperglycaemia, including the prediabetes stage. The authors previously reported an increase in plasma D-dimer in prediabetes compared to healthy individuals.¹⁵ It is known that blood viscosity and plasma D-dimer are two of the three indices of Virchow's triad that indicate vasculopathy and SCVD,¹ and that increased blood viscosity exacerbates atherothrombosis.³

Whole blood viscosity is also increased in prediabetes. It affirms the concept that asymptomatic vasculopathy is present in individuals with prediabetes who are thus at high risk of developing cardiovascular complications.¹⁶ This suggests that determination of blood viscosity in clinical practice may be a useful supplementary tool in the assessment of vasculopathy and SCVD in prediabetes and undiagnosed diabetes. The absence of difference between the DM and DM+CVD groups in the present study shows that oxidative stress risk in DM is as high as that in DM+CVD individuals, which has significant prognostic implications.

A high prevalence of positive EOS indices in abnormal WBV has been demonstrated. This indicates that blood viscosity is never abnormal without underlying physiological cause. Even in apparently healthy people, abnormal WBV could be linked to antioxidant imbalance or oxidative stress in 76% of the population (Table 1). Furthermore, the higher prevalence of positive EOS indices in the three hyperglycaemic groups demonstrates the contribution of hyperglycaemia to increased blood viscosity through EOS, an increase in which leads to increased blood viscosity. The latter leads to diabetic hypercoagulability, which is indicated by increased plasma D-dimer.^{17,18}

A slightly higher prevalence of abnormal EOS indices in the preDM group was observed, compared to the DM and DM+CVD groups (Table 1). This may be due to the minimal antioxidant effects of hypoglycaemic drugs.¹⁹ An almost equal prevalence in the DM+CVD group (91%) compared to the DM group (92%) provides further evidence to support the lack of difference in EOS and therefore viscosity between the two groups. Considering the additional cardiovascular drugs being taken by members of the DM+CVD group, this observation supports earlier preliminary findings that cardiovascular drugs do not significantly alleviate oxidative stress.²⁰

Clearly, this implies that the oxidative stress process of hyperglycaemia-induced vascular pathology requires attention. It has been shown that aspirin therapy is not useful in reducing stasis in the diabetes population,²¹ nor in preventing cardiovascular complications.²² It has also been shown that aspirin therapy has no clinical evidence-base, but, with additional intervention against the underlying oxidative stress, reduces blood viscosity in diabetes.^{21,22} The present study adds evidence that high viscosity varies with different stages of diabetes and is associated with erythrocyte oxidative stress. This simple and cheap measurement could be used to confirm the requirement for, and monitor the effectiveness of, stasis reduction medications in diabetes management.

It is known that glycosylation of haemoglobin reduces the erythrocyte's ability to transport oxygen and can lead to vasculopathy.¹⁸ The findings of the present study are limited

by the non-determination of the contribution from glycosylated haemoglobin (HbA1c). Therefore, further study is required to investigate how different levels of HbA1c affect blood viscosity and its sensitivity to EOS.

In conclusions, increased blood viscosity is present in asymptomatic diabetes and contributes to the pathology of the disease. Thus, determination of blood viscosity may be useful in screening for subclinical vasculopathy in asymptomatic diabetes and early-stage diabetes, in an effort to provide early intervention in unidentified yet progressive subclinical cardiovascular disease. □

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