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Research article

Hemorheological measurements over the shear-thinning regime. In vitro comparative study for hyperglycemia

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Abstract: We investigated the shear thinning of normal and diabetic blood experimentally. The shear thinning of the blood has been analyzed using the Power-law model. Over the viscosity time course, the coagulation process of blood samples from diabetic and healthy subjects was observed. The shear thinning behavior of blood samples was examined in the shear rate ranging from 5 s^{-1} to 222 s^{-1}, and viscosity time-course was studied at a shear rate of 50 s^{-1}. The consistency coefficients were 8.638 ± 0.4860 mPa·s, and 6.658 ± 0.3219 mPa·s for diabetic blood and control, respectively. This difference was statistically significant ($p < 0.0001$). The parameters extracted from the viscosity–time curve were the time-to-gel point (TGP), maximum clot viscosity (MCV), and final clot viscosity (FCV). The diabetic blood exhibited a significantly high ($p < 0.0001$) shorter TGP (148.8 ± 6.024 s) than control (199.1 ± 4.865 s). A considerably higher MCV for diabetic blood (26.39 ± 1.451 cP) than the control (17.54 ± 2.324 cP) was reported. FCV for diabetic blood (10.89 ± 1.12 cP) was significantly higher than control (7.6 ± 0.8 cP). The viscosity time course as well as features obtained via the power-law model reflected the flow state of diabetic blood.

Keywords: blood viscosity; coagulation; gel point; hyperglycemia

1. Introduction

The rheological properties of blood play an essential role in blood circulation along the human body, and there are other factors that control blood circulation, such as myogenic and endothelial factors [1]. Blood viscosity is One of the well-known hemorheological parameters. The whole blood viscosity is altered by many factors, such as plasma viscosity, cell deformability, and cell aggregation under specific hemodynamic conditions [2]. The non-constant whole-blood viscosity is caused by the blood's high cellular content [3,4]. Red blood cells make up the most substantial part of the blood-forming cells, so many of the flowing properties are dependent on them. As the shear rate increases, the whole blood viscosity decreases, and hence whole blood is described as not -Newtonian. The non-Newtonian behavior of the whole blood appears clearly at low shear rates [2,5,6]. Due to the shear-thinning property, blood viscosity cannot be summarized by a single value and should be evaluated under the range of shear rates [7]. There are several models used to describe the non-Newtonian flow of the whole blood. In these models, blood is treated as a single continuum, and various regressions of apparent viscosity introduce shear-thinning to experimental data [4,8].

Diabetes mellitus can affect several hemorheological markers, which are associated with blood flow properties. Diabetes mellitus alters several important hemorheological parameters, such as erythrocyte aggregation, erythrocyte deformability, and whole blood viscosity. Studies have demonstrated that erythrocyte deformability decreases and erythrocyte aggregation increases in diabetes, resulting in elevated blood viscosity in comparison to healthy persons [9,10].

In the context of diabetes, blood viscosity, coagulation, gel point, and hyperglycemia are related. Studies have demonstrated that people with Type 2 Diabetes Mellitus frequently have higher blood viscosities, which can be impacted by hyperglycemia and other variables [11]. Diabetes mellitus can cause hyperglycemia and insulin resistance, which can alter platelet activation, coagulation factors, and fibrinolysis [12]. These changes can culminate in a prothrombotic condition that is marked by decreased fibrinolysis and enhanced coagulation. Additionally, it has been discovered that hyperinsulinemia can restrict fibrinolysis, while hyperglycemia can improve coagulation and decrease neutrophil degranulation [12,13]. These effects play an essential role in the impaired coagulation and hemostasis most patients experience [14]. Studies on the connection between blood viscosity and blood glucose levels have revealed a direct correlation between the two in both diabetic and non-diabetic participants [15]. Additionally, research has been done on the effects of hyperglycemia on blood pressure and viscosity. The results indicate that hyperglycemia may raise blood viscosity, necessitating an increase in blood pressure to maintain sufficient blood flow. Thus, especially in the setting of diabetes, hyperglycemia has a major impact on blood viscosity, coagulation, and hemostasis [16,17].

We evaluated how blood viscosity changed at various shear rates during the coagulation process to find characteristics that may be helpful for clotting time, clot hardness, and clot stability for diabetic blood.

2. Materials and methods

2.1. Sample collection and preparation

This study was conducted following the requirements of the ethics committee of the Medical Research Institute. Alexandria University (Potocol serial number: E/C. S/N. 09/2020). 25 patients with

type 2 diabetes mellitus (males, aged 40.6 ± 5 years, mean duration of diabetes 5.6 years) were recruited for this study. They were compared to 20 healthy volunteers (males, aged 42.2 ± 5 years). Two ml of blood were collected in a tube containing sodium fluoride and sent to a medical laboratory for plasma glucose testing. Two ml of blood were collected using ethylenediaminetetraacetic acid (EDTA) as an anticoagulant and used for power-law coefficient analysis. Two ml of blood were collected in a blind tube and used for viscosity time-course analysis immediately after withdrawal.

2.2. Power-law coefficients analysis for blood viscosity

Apparent blood viscosity (BV) was measured using a cone-plate viscometer (Brookfield, CAP 1000+ ™ & CAP 2000+ ™). All viscosity measurements were performed at a constant temperature of 37 °C and shear rate range of 5 s^{-1} to 222 s^{-1}. The shear rate range was chosen to cover the shear-thinning regime of the blood. The power-law model for the viscosity (η) is expressed as follows:

$$\eta = m\gamma^{n-1} \tag{1}$$

where m is often known as the consistency coefficient (mPa.s), γ sear rate (s^{-1}), and n is the flow index. Moreover,

$$\log \eta = \log m + (n-1)\log \gamma \tag{2}$$

where m is often known as the consistency coefficient (mPa.s), γ sear rate (s^{-1}), and n is the flow index. The measured BV corresponding to the shear rate was plotted against the adjacent shear rate on thelog–log scale. The slope of the line obtained from the plot is equal to the power-law exponent (p) and the intercept with the y-axis is equal to ($\log m$). Hence, p and m were calculated.

2.3. Viscosity time-course analysis for blood coagulation

Immediately after blood collection, the whole blood was examined using a cone-plate viscometer. Blood (300 μL) was added to the plate surface. Viscosity measurements were performed at a shear rate of 100 s^{-1} at a constant temperature of 37 °C. The viscosity was recorded every 50 s. The time course of viscosity measurement was between 1 and 5 min, depending on the time required to estimate the extracted parameters from the time–viscosity curve. The smoothing technique was applied using GraphPad Prism 8.0. Moreover, an approximation function for the graphed data is generated. Graphical analysis was performed by approximating the function during blood coagulation. The following parameters were extracted from the graphical analysis of the viscosity–time curve[18−20]:

- The time to gel point (TGP) was defined as the time required for the blood to be transformed into a gel-like state.
- The maximum clotting viscosity (MCV) is defined as the maximum blood viscosity in the viscosity time graph.
- The final value of viscosity (FCV) was defined as the value of blood viscosity in the plateau region of the viscosity time graph.

2.4. Statistical analysis

Statistical analyses were performed using GraphPad Prism 8.0 for Windows. Continuous

variables were compared using nonparametric tests and investigated relationships between viscosity-related factors and coagulation tests using regression analysis. All parameters are represented as mean ± SD. The t-test was used to evaluate the statistical difference between the measured parameters for diabetic and control blood samples.

3. Results and discussion

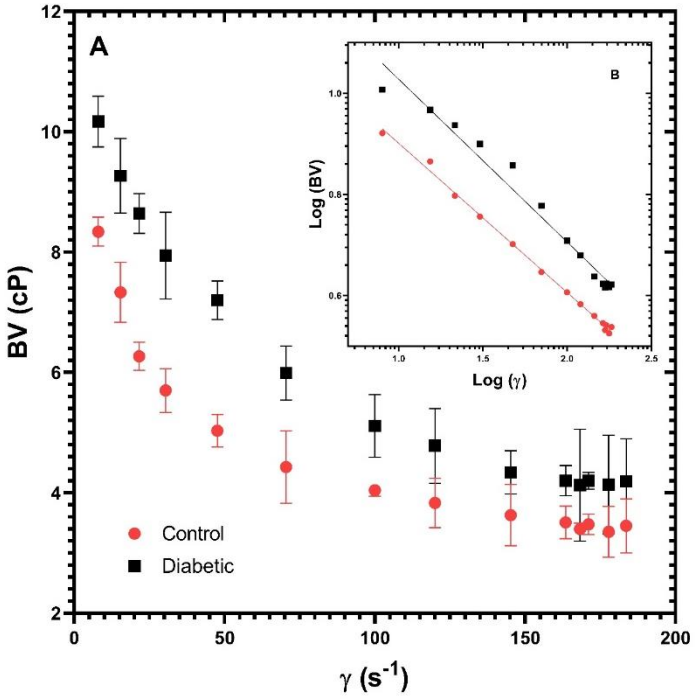

Figure 1. Blood viscosity versus shear rate. A. The plot addresses the shear-thinning of control and diabetic blood in the investigated shear rate range. B. Blood viscosity versus shear rate in the log-log scale. The obtained lines were used to calculate the power-law exponent and the flow consistency coefficient.

The blood viscosity decreases with the shear rate, which is called shear thinning, and blood shear thinning is initiated by red blood cells, which are the major constituents of the blood [21]. A comparison between the shear thinning of diabetic and normal blood was performed under physiologically relevant conditions of both flow and viscosity. A power-law model was used to describe the shear-thinning behavior of the blood, as shown in Figure 1. Diabetic and control blood samples showed comparable shear-thinning behavior with different BV values. A significant difference between the BV of diabetic blood compared to that of the control was observed ($p < 0.0001$). Diabetic blood showed higher viscosity than healthy controls. The experimental results obtained fitted well with the power-law model for both the control and diabetic blood. This finding follows previous studies, which showed that the most relevant models to represent blood rheology are non-Newtonian pseudoplastic power fluids [22−24]. Several studies suggested that an increase in BV may lead to the development of microvascular complications [25,26]. Concetta Irace et al. reported a significant increase in BV in the diabetes group, with blood glucose ranging from 100 to 125 mg/dl. They obtained BV of 4.9 ± 0.7 mPa.s at shear rates of 225 s^{-1} and 8.41 ± 2.51 mPa.s at a shear rate of 22 s^{-1} for the

mentioned blood glucose level [27,28]. A significant increase in blood viscosity has been previously reported in diabetic patients [14]. BV measured in the present study is following previous studies for diabetic and control blood. This confirms that hyperviscosity is expected in patients with diabetes.

Power-law model coefficients (P, m) were calculated from the BV plot versus the shear rate on a log-log scale as shown in Figure 2B. M for the control group was 6.66 ± 0.32 mPa·s, whereas that for diabetic blood was 8.64 ± 0.49 mPa·s, and the difference between them was highly significant ($p < 0.0001$). P for control was -0.017 ± 0.003, while for diabetic blood, it was -0.025 ± 0.003, and the difference between them was not statistically significant. Several models have been used to describe the shear-thinning behavior of the blood. Shear-thinning in these models is represented by various regressions of apparent viscosity, such as logarithmic and power-law functions [1,8]. Power law regression has been proven suitable for hemodynamics in normal and pathogenic cases [29,30]. Nadia A. reported that the power-law coefficients (m, n) for healthy control blood are 13.18 ± 5.25 mPa.s and 0.79 ± 0.07 mPa.s, respectively [4]. Panagiotis used the power-law model with m = 14.67 and n = 0.78 in his computational model for blood flow simulation. Hussain et al. mentioned that n = 0.71 and m = 16.5 mPa.s for normal blood [30]. These alterations occur due to changes in the concentrations of some blood-forming chemicals, such as glucose [31,32]. In the present study, the viscosity of the diabetic blood was higher than that of the control; accordingly, the power-law coefficient values differed from those of the control.

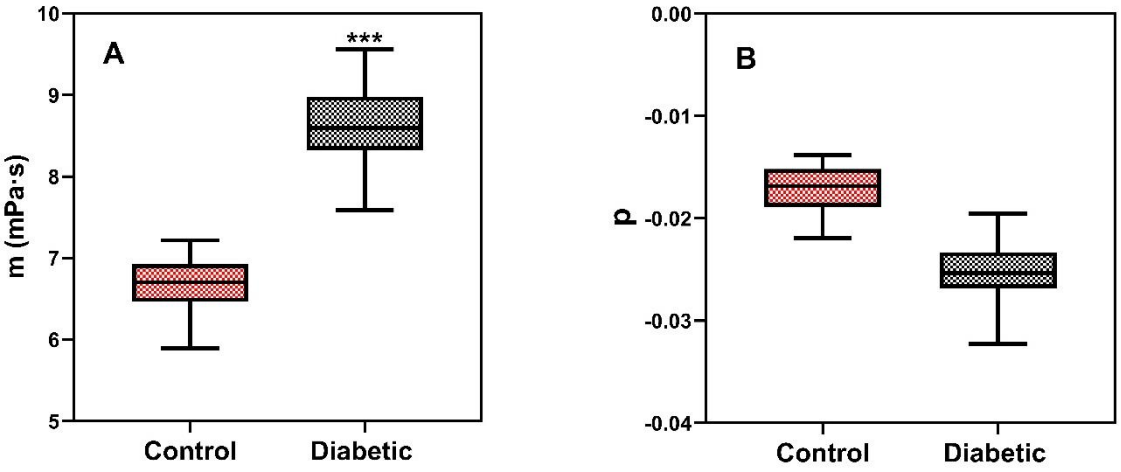

Figure 2. A. Flow index computed from the power law. B. Flow consistency index computed from the power law. *** is considered to be highly statistically significant ($p < 0.0001$).

Figure 3 (A) shows the experimental procedure for monitoring clot formation in the present study. Figures 3 (B) and (C) represent the Lowess curve generated to smooth the scatter plot of the viscosity time course for the control and diabetic blood. The diabatic blood exhibited a significantly ($p < 0.0001$) shorter TGP (148.8 ± 6.02 s) than the control (199.1 ± 4.86 s). A significantly higher MCV for diabetic blood (26.39 ± 1.45 cP) than the control (17.54 ± 2.32 cP) was reported. FCV for diabetic blood (10.89 ± 1.12 cP) was significantly higher than control (7.61 ± 0.82 cP). Figures 4 (A), (B), and (C) show the comparison between TGP, MCV, and FCV for diabetic blood and control.

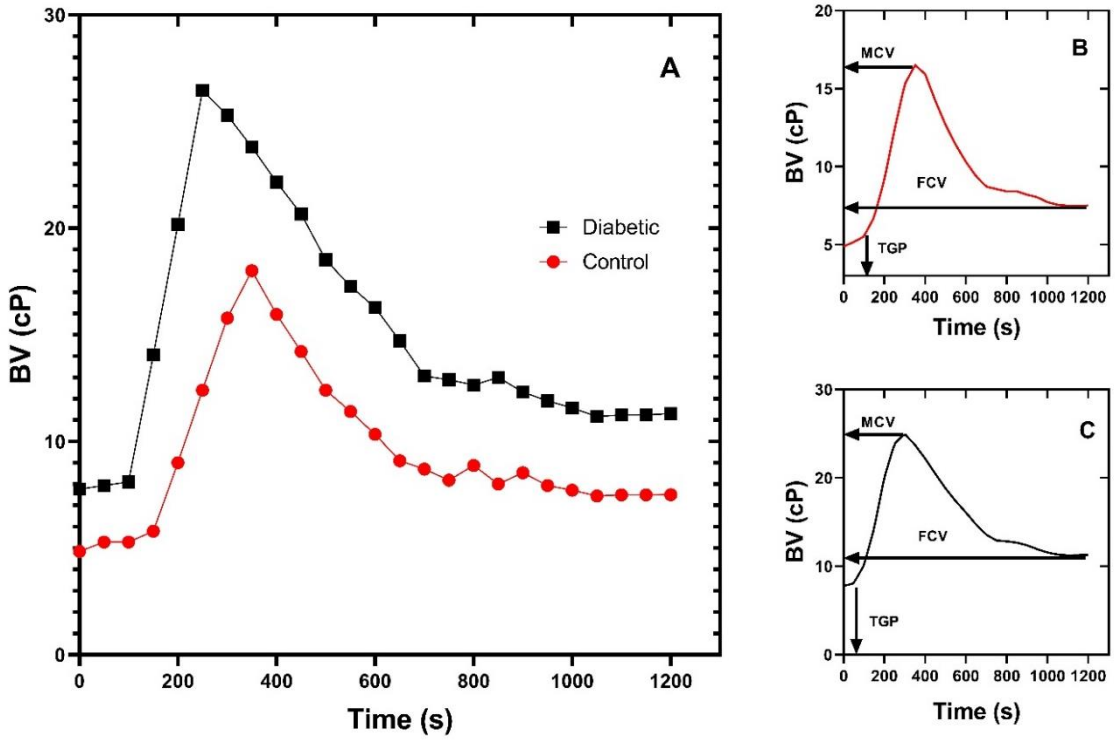

Figure 3. (A) Experimental results of the viscosity time-course during blood coagulation at a shear rate of 25 s^{-1}. (B) scatter plot smoothing for control data (C) scatter plot smoothing for diabetic blood data.

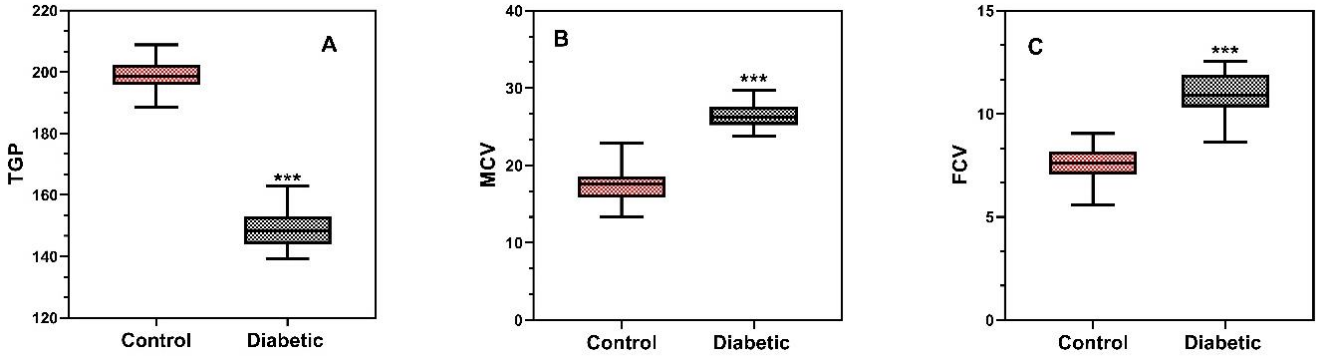

Figure 4. (A) Time to gel point for control and diabetic blood. (B) Maximum clotting viscosity for control and diabetic blood. (C) Final clotting viscosity for control and diabetic blood. The data reparented as mean ± SD and *** is considered to be highly statistically significant ($p < 0.0001$).

Our results indicated that the viscosity time-course measurement can represent the coagulation process for normal and pathological conditions under specific flow conditions. The suggested parameters demonstrate different stages of clot formation and lysis. Beginning at the TGP, the transformation of blood to a gel-like form is indicated, and this stage depends on the intrinsic pathway activation of blood. MCV and FCV reflect the stage after clot formation or clot lysis. Therefore, this experimental procedure, which was previously suggested by [11,13], could be used to monitor diabetic

blood coagulation. Clotting time is an essential clinical parameter associated with thromboembolic events or bleeding. Thromboembolic events appear in prothrombotic diseases and increase coagulation factors, similar to diabetes [33,34]. Evans et al. defined the gel point as a marker of incipient clot formation [20]. Marco Ranucci et al. studied the relationship between TGP and activated partial thromboplastin time (aPTT) at different shear rates. They found a significant association between TGP and aPTT at shear rates of 20 s^{-1} and 80 s^{-1} [35]. In the present study, the TGP of diabetic blood was significantly shorter than that of the control, which is relevant as diabetes may increase coagulation activity. The TGP represents the clotting time well and can be used as a biomarker, especially for diseases that cause blood clotting. MCV of the diabetic blood was higher than that of the control group. In addition, diabetic blood reached the MCV more rapidly than the control blood. The viscosity time course for the majority of the samples used in this study reached a steady state (plateau) for both the control and diabetic blood. FCV of diabetic blood was higher than that of the control and the starting BV values before gel point formation.

4. Conclusions

The study provides insights into the rheological properties of diabetic blood and its potential implications for the evaluation of hemorheological complications associated with hyperglycemia. Despite the hemorheological disturbance accompanying diabetes mellitus, diabetic blood exhibits shear thinning as well as normal blood. The power-law coefficients obtained in the study are extremely useful for understanding the rheological behavior of diabetic blood and could be used to differentiate between the blood in the normal and abnormal state. The viscosity time course analysis could be a useful tool to monitor coagulation at different stages.

Use of AI tools declaration

The authors declare that they have not used Artificial Intelligence (AI) tools in the creation of this article.

Conflict of interest

The authors declare no conflicts of interest.

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