Whole Blood Viscosity in Beta Thalassemia Minor

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ABSTRACT

Patients with heterozygous β-thalassemia minor have a decreased hematocrit (HCT). Since the HCT is a primary determinant of whole blood viscosity, the known reduction in HCT in β-thalassemia minor should lead to a measurable reduction of whole blood viscosity. The influence of the relatively lower mean corpuscular volume and consequent higher red blood cell count and β-thalassemia minor on whole blood viscosity using a microporous viscometer has not previously been the subject of investigation. Accordingly, the blood of a group of normal and β-thalassemia minor subjects was examined with a microporous viscometer to elucidate further the relations between whole blood viscosity, HCT, and red blood cell count. The data show that for normal and β-thalassemia minor subjects a significant positive correlation (r = 0.65, p < 0.01) exists between HCT and whole blood viscosity. However, the slope of the regression of whole blood viscosity and HCT of β-thalassemia minor subjects was significantly higher (z = 3.14, p < 0.001) than that of normals. Thus, for any given HCT their whole blood viscosity was higher than that of normals. Studies of the rela-
tation of red blood cell counts to whole blood viscosity indicate the higher whole blood viscosity at a given HCT was related to the increased red blood cell counts in β-thalassemia minor subjects. Because of the opposing interactions of HCT and red blood cell counts, the mean whole blood viscosity of the group of β-thalassemia minor subjects examined was not significantly lower than the normal whole blood viscosity. In fact, β-thalassemia minor subjects with HCT > 42, had a whole blood viscosity significantly higher (p < 0.001) than normals with HCT > 42.

Introduction

A recent study of hospitalized patients has demonstrated a lower than expected incidence of β-thalassemia trait in patients admitted to the hospital with myocardial infarction. Since mild anemia is often present in subjects with β-thalassemia minor, one explanation for this finding is an ameliorative effect of a lower hematocrit. A beneficial effect of mild anemia on the atherosclerotic microcirculation might be related to lowered whole blood viscosity since a lower whole blood viscosity would lead to improved blood flow in vessels where resistance to flow has increased.

The relation between HCT and whole blood viscosity has been studied previously. The HCT may be used to estimate whole blood viscosity because the relative viscosity of blood to water varies approximately as the HCT is raised to the power of 1.3. However, since the viscosity of blood increases as the velocity of flow decreases owing to the aggregation of red blood cells, Guyton suggested that clinically relevant measurements of whole blood viscosity require an instrument with measuremental characteristics resembling the low shear rates and shear stress of the microcirculation.

A porous sintered polyolefin viscometer has been developed with a low shear rate and shear stress for clinical measurement of whole blood viscosity.

Using this instrument, the whole blood viscosity was measured of 31 adults including 16 subjects with β-thalassemia minor to determine if the whole blood viscosity in β-thalassemia minor subjects is actually reduced.

Materials and Methods

BLOOD SAMPLES

Fifteen to 20 ml of blood were drawn into a syringe by venipuncture from each individual studied. Seven ml were placed in a tube containing ethylenediaminetetraacetic acid (EDTA) and reserved for hematologic measurements. The remaining blood was immediately used to measure viscosity. An additional 30 ml of blood was drawn on some individuals and mixed with 4.3 ml anticoagulant citrate phosphate dextrose solution to be used for the preparation of saline-suspended red blood cells.

SUBJECTS

Thirty-one individuals were studied. Fifteen of these subjects were normal, healthy hospital personnel with no history of anemia and normal HCT values. Only microhematocrits were performed. The remaining 16 individuals were all previously diagnosed by hemoglobin electrophoresis as having thalassemia trait. Thirteen of the 16 had elevated hemoglobin A2 levels (8.3 ± 3.1) available through hospital records. The

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remaining three had been diagnosed elsewhere years ago and the actual A2 level was unavailable. However, each of these three had a positive family history for β-thalassemia minor, microcytosis and anemia. The first eight β-thalassemia minor subjects were tested only by microhematocrit. The last eight individuals had Coulter counts performed, including red blood cell counts and mean corpuscular volume.

**Viscosity Determination**

Viscosity measurements were made with a microporous viscometer which is a disposable device composed of a cylindrical plug of sintered polyolefin connected to a capillary tube above and a fitting for connection to a three way Luer lock stopcock below.

The microporous polyolefin plug is characterized by a Darcy permeability ($B_o$) of about 8 Darcy ($8 \times 10^{-8} \text{ cm}^2$) as determined by calibration with three standard silicone calibration oils ranging from five to 20 centipoise and the use of the defining equation:

$$Q/A = (B_o/\mu) (\Delta P/L)$$

where $Q = \text{cm}^2/\text{sec}$ of fluid flow toward plug;

$A = \text{total cross sectional area of plug (solid included) normal to approaching flow, cm}^2$ (fixed);

$\mu = \text{fluid viscosity, in poise (dyn/sec/cm}^{-2}$ (variable);

$\Delta P = \text{pressure drop due to hydrostatic head (fixed), dyn/cm}^{-2} = \rho g \Delta H_{avg}$.  

Thus, with $\Delta H_{avg} = 13.3 \text{ cm (the mean hydrostatic head in the capillary tube)}$, $g = 980 \text{ cm/sec}^2$, and density $\rho = 1.04 \text{ g/cm}^{-3}$ (blood, blood plasma, silicone calibration oil), $\Delta P$ is found to be $2.8 \times 10^3 \text{ dyn/cm}^{-2}$. The flow time, in seconds, was found to be approximately 4.0 times the viscosity in centipoise, and, as expected, was linearly proportional to the viscosity of the calibration liquid. Variation in flow times between viscometers with the same calibration fluid was less than ±5 percent.

These conditions lead to the result that the effective shear stress in the pores, around one dyn/cm$^{-2}$ or less, will allow blood to flow slowly enough to reveal its non-Newtonian properties, since red cell interactions mediated by fibrinogen will be significant.

The mean diameters of the communicating channels of the porous bed, are within a range relevant to arterioles and venules ($\approx 40 \mu\text{m}$). Moreover, the low pressure gradient across the bed, in combination with its permeability and the slow flow rate simulates flow in the microcirculatory vessels. The mean shear stress, about one dyn per cm$^2$, is fixed by the meniscus height, the bed cross section, and the pore diameter. The flow rate adjusts itself according to the tendency of the red cells to interact.

To measure viscosity at room temperature, three microporous viscometers are mounted vertically, and approximately 10 ml of blood are drawn by venipuncture into a syringe without anticoagulation. The needle is removed from the syringe, and any air bubbles in the syringe are expelled. The syringe is connected to the three-way stopcock at the base of the first microporous viscometer, and the blood is gently injected into the viscometer, flowing upwards through the porous bed and into the capillary tube. When the meniscus of the blood is above the upper mark on the tube, the syringe is removed, and the stopcock arm is raised to 90° permitting the blood to flow downwards by gravity through the viscometer.

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A stopcock watch is started when the meniscus reaches the upper mark, and the time recorded when the meniscus reaches the lower mark on the capillary tube. The measurement is done in triplicate using a new microporous viscometer for each measurement, and the mean flow time is recorded as the estimate of viscosity.

Hematologic Parameters

Decreased hematocrit was measured either by a standard high speed microhematocrit method or by a Coulter Counter, Model S.* Whole blood red blood cell counts and mean corpuscular volume were also measured by a Coulter Counter, Model S.* Saline suspensions or red blood cell counts were counted by a standard method on a Coulter Counter, Model ZB1.*

Preparation of Saline Suspended Red Blood Cell Counts

Citrate phosphate dextrose solution, anticoagulated blood was washed three times by centrifugation in a saline solution containing 0.9 percent NaCl, 0.2 percent dextrose, 40 mg percent inorganic phosphorus, pH 7.0 with an osmolality of 340 mOsm per kg. After each centrifugation, care was taken to remove all buffy coat visible. Sufficient saline solution was added to produce a suspension with either a HCT of 0.60 or a $6.0 \times 10^{12}$ per ml red blood cell counts. For each suspension, both microhematocrit and red blood cell counts were performed. Viscosity was measured in duplicate on each sample.

Statistical Analysis

The mean and standard deviation (SD) or standard error (SE) of the mean was reported for each group in each type of experiment. The means of the two groups were compared using the non-paired t-test. Linear regression, correlation coefficient, analysis of covariance, and the paired t-test were used to measure the relationship between whole blood viscosity and a variable (e.g., HCT). Slopes were compared by dividing the arithmetic difference between the slopes by the confidence limits. A z value of >2.0 was considered significant. Certain measurements were performed only on some individuals. The data indicate the number (N) of samples used for each determination. A p value of <0.05 was considered significant for all tests.

Results

There was no significant difference between the whole blood viscosity of normal subjects when compared to that of β-thalassemia minor subjects by the independent t-test (Normal = 34.1 ± 6.3 sec., vs. β-thalassemia minor = 40.0 ± 14.3 sec.). There was a significant correlation ($r = 0.65$, $p < 0.01$) between HCT and whole blood viscosity overall (figure 1). However, it was apparent that most of the β-thalassemia minor points lie to the left of the calculated regression line, and most of the normal points lie to the right. There was a significant difference ($z = 3.14$, $p < 0.001$) between the slopes of the regression lines that can be calculated for each of the groups which indicates that whole blood viscosity rises more as a function of HCT in β-thalassemia minor subjects than normals. Thus, if the β-thalassemia minor and normal subjects are divided into two groups, those with a HCT < 42 and those with a HCT > 42, the mean of β-thalassemia minor subjects with HCT > 42 is significantly higher ($p < 0.001$) than that of normals (figure 2).

A strong correlation ($r = 0.9$, $p < 0.001$) was seen between whole blood viscosity and red blood cell counts in β-thalassemia minor and normal subjects. The data indicate the number (N) of samples used for each determination. A p value of <0.05 was considered significant for all tests.
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PCV (Volumes %)

FIGURE 1. The relation between hematocrit (packed cell volume) and whole blood viscosity measured by the microporous viscometer for 15 normal and 16 β-thalassemia minor subjects.

β-thalassemia minor suspensions had a significantly lower viscosity than did the normal suspensions (figure 4).

Discussion

Under conditions of low shear rate and shear stress, the whole blood viscosity of a group of β-thalassemia minor subjects was not significantly different from that of normal subjects (β-thalassemia minor = 40.0 ± 14.3 sec., Normal = 34.1 ± 6.3 sec.), and at HCT > 42 it was significantly higher. The whole blood viscosity of subjects with β-thalassemia minor has been studied previously by other techniques. Stasiw et al. used a Wells-Brookfield cone plate viscometer and also found no major difference in whole blood viscosity between five β-thalassemia minor subjects and six normal subjects. Our results are consistent with these reports since there is no significant difference between whole blood viscosity of normal and β-thalassemia minor subjects.
Tillman and Schroter measured the viscosity of erythrocyte suspensions prepared from the blood of 12 β-thalassemia minor subjects at a fixed HCT of 80 with a Wells-Brookfield viscometer at shear rates between 0.39 and 78.6 sec.\(^{-1}\) and found the values to not be significantly different from normal. In contrast, a significantly higher whole blood viscosity was found by us (figure 3) when β-thalassemia minor red blood cells were suspended in saline at a HCT of 60. As discussed in detail in our recent report on the microporous viscometer, there are significant methodologic differences between the microporous viscometer and cone plate viscometers. It is likely that the branching capillary bed of the microporous viscometer makes it more sensitive to the higher red blood cell counts that result from the low mean corpuscular volume in β-thalassemia minor than the flat surface of the cone plate viscometer.

Strumia studied the effect of red cell factors on the relative viscosity of whole blood using an Ostwald U-tube viscometer. He found that the relative viscosity of whole blood measured this way was influenced by the red cell size: when the red blood cell count was held constant, microcytosis was accompanied by a decrease of the relative viscosity of whole blood while macrocytosis was accompanied by an increase. The difference between our results and that of Strumia is probably explained by the experimental method, since the Ostwald viscometer has a very high shear rate relative to the viscometer used by us. Why the size of the red cell would have the opposite effect on blood studied in a high shear rate versus a low shear rate is unknown and is deserving of further study. As discussed, our results are consistent with the more recent reports of the whole blood viscosity in β-thalassemia minor since no significant difference between whole blood viscosity of normal and β-thalassemia minor subjects was observed by us.

FIGURE 3. The relation between viscosity and red blood cell counts for six normal and 11 β-thalassemia minor subjects after the red blood cells were washed and resuspended in buffered saline at a hematocrit of 60.

FIGURE 4. A comparison of the viscosity of the washed, saline suspended red blood cells of β-thalassemia minor and normal subjects.
While the mean whole blood viscosity of β-thalassemia minor subjects was no different from normal, the correlation between HCT and whole blood viscosity was significantly different from that of normal (figure 1, Results). For any given HCT, there was a higher whole blood viscosity in β-thalassemia minor than that predicted from normals. The lower mean corpuscular volume in β-thalassemia minor causes a higher red blood cell count than normal at any given HCT, which suggests that the red blood cell count itself may independently influence the whole blood viscosity in β-thalassemia minor. When normal and β-thalassemia minor erythrocytes were each suspended to a fixed HCT of 60, a highly significant difference in viscosity was demonstrated between β-thalassemia minor and normal (p < 0.001), thus confirming the independent influence of red blood cell counts on whole blood viscosity. The increase in whole blood viscosity owing to the higher red blood cell counts at any given HCT explains the failure to observe the expected reduction in whole blood viscosity for β-thalassemia minor.

A note on technique seems worth mentioning. The measurement of whole blood viscosity with the microporous viscometer was a relatively simple device to measure whole blood viscosity when compared to other existing methods. Moreover, the viscometer was sensitive to alterations other than HCT (viz red blood cell counts) that influence whole blood viscosity. Thus, the measurement of whole blood viscosity with the microporous viscometer deserves further study in settings in which dysproteinemias or extreme leukocytosis alter the usual and expected relation between the HCT and the whole blood viscosity.

The results of this study suggest that a decrease in whole blood viscosity does not seem a likely explanation for the prior observed reduction in the occurrence of myocardial infarction in subjects with β-thalassemia minor. An alternative suggestion that the reduced incidence of myocardial infarction in β-thalassemia minor may be due to the lower cholesterol levels needs further investigation. Studies of high and low density beta lipoproteins or fractionation of the apolipoproteins would therefore be of special interest in β-thalassemia minor in view of the predictive value of these measurements for the development of atherosclerosis.

References