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Full Length Research Paper

Rheological Variations among Nigerians with Different Hemoglobin Genotypes

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ABSTRACT

A total of one hundred and Forty-five (145) subjects comprising of 50 homozygous Hemoglobin A subjects (HbAA), 50 heterozygous hemoglobin AS (HbAS) subjects and 45 homozygous hemoglobin S (HbSS) subjects were recruited for this study with a view to ascertain variations in the Hemorheological values possibly associated with the inherited hemoglobin genotype. Some Hemorheological determinants such as whole blood viscosity (WBV) and plasma viscosity (PV) and Plasma Fibrinogen Concentration (PFC) were measured with standard methods. We recorded a relatively unchanged whole blood viscosities in subjects with various hemoglobin genotypes (AA, AS and SS; $P>0.05$, respectively). Also, there were no significant differences in PV values of HbAA and HbAS while there were significant increases in PV and PFC of HbSS compared with others ($P<0.05$, respectively). However, relative erythrocyte viscosity (REV) of HbSS became significantly reduced when repeated with saline after replacing plasma with saline ($P<0.05$, respectively), to ascertain the erythrocytic cellular viscosity. We conclude that increased plasma viscosity coupled with that of PFC in HBSS could be due to plasmatic components and that cellular rheologic properties of the erythrocytes may be dependent on its content of hemoglobin while whole blood viscosities are stable in native blood irrespective of haemoglobin genotypes.

Key words: Hemorheology, Plasma Viscosity, erythrocyte cellular viscosity, hemoglobin genotype

INTRODUCTION

Hemorheology is concerned with the dynamics of deformation and flow properties of cellular and plasmatic components of blood, these contributes to the physiology and pathology of blood circulation. The diversity and complexity of these phenomena provides stimulating challenges to the development of the experimental techniques, the underlying theories, the diagnostic test and the treatment procedures (Adelia, 2005). Viscosity of blood reflects its rheological properties which can be influenced by hematocrit, plasma viscosity, red cell aggregation and red cell deformability. Plasma viscosity with all these factors, depends primarily on the concentration of plasma proteins especially fibrinogen (Lewis, 2002; Ajayi *et al*., 2005).

Sickle cell anaemia is a severe hemolytic disorder caused by the homozygous occurrence of the abnormal S hemoglobin (HbSS). It arises from a single point mutation of adenine to thymine, which results in substitution of valine for glutamic acid at the sixth

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position of the beta-globin chain (Famodu *et al*., 1998; Halim *et al.*, 2001). The resultant abnormal hemoglobin (sickle hemoglobin) deforms the red blood cells when they are under low oxygen conditions. Studies have shown that deoxyhemoglobin forms a gel and subsequently, polymers. These hemoglobin polymers are arranged in form of microfibrils, within the red cell and this disorts the cell from its normal spherical shape to a sickle shape with pointed ends. The sickle cell formed get blocked in capillaries because they are not flexible. A critical point of sickle cell anemia (SCA) is represented by rheologic alterations of sickle cells determined by the transition from sol to gel of haemoglobin producing a dramatic change in cell viscosity and viscoelastic properties. Decreased deformability of sickle cells has been documented using varieties of techniques (Dobbe *et al*., 2002; Handerman *et al*., 1999), their reports have pointed out an increased viscosity of blood, decreased filtration rate of diluted cell suspension through narrow pores, decreased ability of cells to undergo deformation in shear fields, and increased aspiration pressure needed for movement of cells into microvessels. Previous studies have sought for different mechanisms on hemorheology on sickle cell patients, but have not exactly compared it on their different hemoglobin genotypes. We aimed to compare the rheologic properties of the blood with different hemoglobin genotypes in this study to express views on the roles of the hemoglobin content and type in determining the erythrocyte flow properties.

MATERIALS AND METHODS

A total of one hundred and Forty-five (145) subjects comprising of 50 homozygous Hemoglobin A subjects (HbAA), 50 heterozygous hemoglobin AS (HbAS) subjects and 45 homozygous hemoglobin S (HbSS) subjects were recruited for this study. Patient's verbal and informed consent as well approval from sickle cell Centre, Benin City were duly obtained for the study. Hemorheological determinants such as whole blood viscosity (WBV) and plasma viscosity (PV) and Plasma Fibrinogen Concentration (PFC) were measured with standard methods.

Ten milliliters (10ml) of blood was collected from cubital vein and divided appropriately into 0.5ml of 3.8% sodium citrate in a plastic containers and Ethylene Diamine Tetra acetic acid (EDTA) plastic containers. The blood collected into sodium citrate plastic tube was centrifuged immediately and the plasma separated and stored into stopper tubes and used within 3 hours of collection for plasma fibrinogen concentration (PFC). The EDTA blood sample was used for RPV, RWBV and REV estimations respectively.

The simple viscometer technique, described by Reid and Ugwu, (1987), based on the rate of flow used for viscosities while the Gravimetric Assay method as adapted by Mackie and Machin, (1989) was used for PFC. Briefly, the fluid to be tested was drawn up into the vertical syringe, taking care to avoid air bubbles, until the end of the plunger passed the 1ml graduation mark. The plunger was then removed carefully, and a stop-watch started when the lower meniscus of the fluid fell to the 1ml graduation mark. The time required for 1ml of fluid to flow down the syringe was noted. The same syringe and needle combination was used for the whole series of measurements. The relative whole blood and plasma viscosities, and then erythrocyte in saline viscosity were expressed as the ratio of the flow-time for 1ml of test specimen to the same volume of distilled water.

Relative viscosity was calculated as:

RWBV = $\frac{Tx}{xTw}$ where, Tx – time of flow flow of test specimen and Tw time of flow of water.

Statistical analysis

Data were analyzed with Microcal origin 5.0 statistical software. The student t-test was used for data comparison. A $p<0.05$ was considered significant.

RESULTS

The Relative whole blood viscosities in different Haemoglobin genotypes are shown in Figure 1. There was no statistical significant differences in the mean values of Hb AS, AS and SS genotypes (P>0.05, respectively)

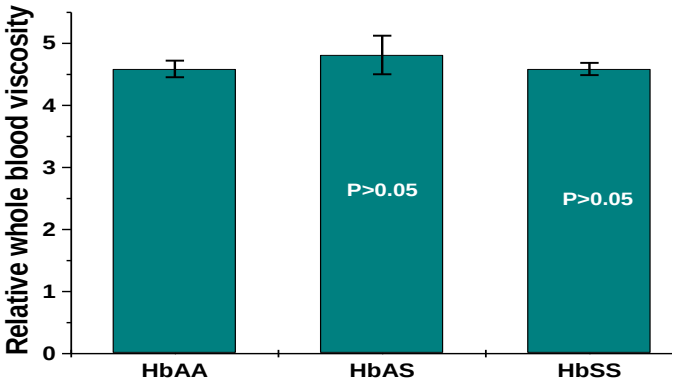

Fig 1:
RWBV showed no significant genotypic differences in HbAS, HbSS and (HbAA) (P>0.05, respectively).

Figure 2 shows the relative Plasma viscosities in different Haemoglobin genotypes. There was a significant increase in the mean RPV value of HbSS subjects compared with HbAA and HbAS (P<0.05, respectively) while HbAS and HbAA showed no

significant differences between each other (P>0.05). This indicate an elevated cellular rheology in the sickle cell haemoglobin due to some intrinsic factors.

The relative Erythrocyte viscosity after plasma was replaced with normal saline is shown in Figure 3. REV showed a significant decrease in the erythrocytes of HbSS subjects compared with HbAA and HbAS (P<0.05, respectively), but no significant difference was observed in HbAS when compared with HbAA (control) (P>0.05). This associates some plasmatic factors influence in the elevated erythrocyte viscosity of the Hb SS subjects.

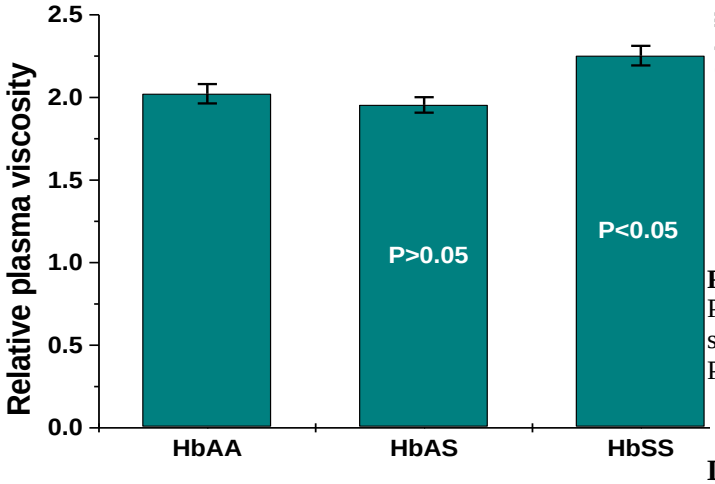

Fig. 2:
RPV showed a significant increase in HbSS patients compared with HbAA and HbAS (P<0.05, respectively), but no significant difference was observed between HbAS and HbAA (P>0.05).

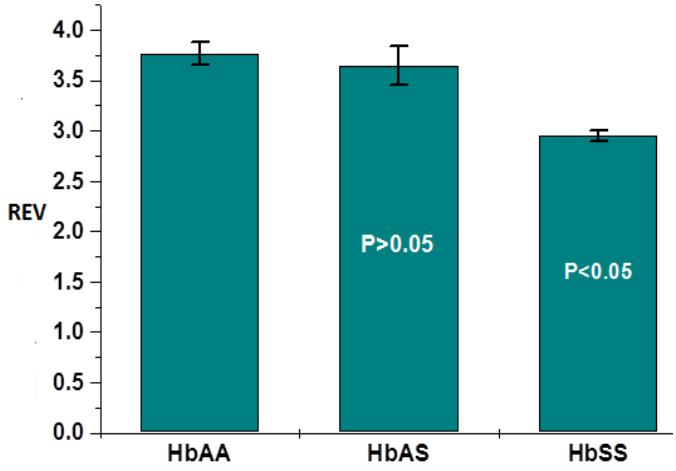

Fig 3:
REV showed a significant decrease in HbSS subjects compared with HbAA and HbAS (P<0.05, respectively), but no significant difference observed in HbAS when compared with HbAA (control) (P>0.05).

Figure 4 shows the plasma Fibrinogen Concentration in different Haemoglobin genotypes. There was a statistical significant increase (P<0.05) in the PFC value of HbSS compared with HBAA and HbAS. This increase seems to be an intrinsic feature of the HbSS subjects being under continuous inflammatory state.

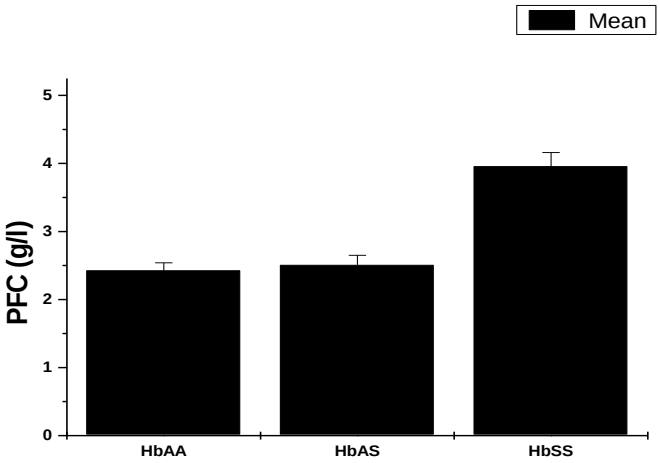

Fig 4:
PFC in the different haemoglobin genotypes. There was a statistical significant increase (P<0.05, respectively) in the PFC value of HbSS compared with HBAA and HbAS.

DISCUSSION

The viscosity of oxygenated blood from patients with sickle cell anemia (HbSS disease) was found to be abnormally increased, a property which contrasts with the well-recognized viscous aberration produced by deoxygenation of HbSS blood. Experiments designed to explain this finding led to considerations of deformation and aggregation, primary determinants of the rheologic behaviour of erythrocytes as they traverse the microcirculation. Deformability of erythrocytes is in turn dependent upon internal viscosity (i.e. the state and concentration of hemoglobin in solution) and membrane flexibility (Chien, 1987). Definition of the contribution made by each of these properties to the abnormal viscosity of oxygenated HbSS blood was made possible by analysis of viscosity measurements, made over a wide range of shear rates and cell concentrations, on HbSS erythrocytes and normal erythrocytes suspended in Ringer's solution (where aggregation does not occur) and in plasma.

We have tried to mimic erythrocyte viscosity measurements by re-suspending the cells in normal saline to eliminate the plasmatic effects in different hemoglobin genotypes. Our observations showed no significant changes in relative whole blood viscosities between subjects with various hemoglobin genotypes

(AA, AS and SS). These observations are at variance with the findings of (Rosenson and Tangney, 1998), where a raised whole blood viscosity (WBV) was observed with HbSS compared with controls HbAA. A possible explanation for this may be the imbalance in haematocrit (Hct), we used the native samples for our experiments, whereas, Hct was corrected for in the previous studies. Also, the internal viscosity of sickle red cells are known to be very high compared with that of normal haemoglobin (Shin *et al*., 2005); hence the contributions of sickle cell hemoglobin in both HbAS and HbSS may have increased the overall viscosities in both cases despite the low Hct values especially for HbSS. Though, there were no significant differences in the HbSS blood viscosity compared with HbAA and HbAS in our study, there was an observable experimental increase.

Our results with plasma viscosities in the various categories of Hb genotypes indicated relatively stable values between HbAA and HbAS but significantly increased with HbSS ($P<0.05$). The plasma viscosity determinants seemed stable in these cases i.e. factors such as plasma fibrinogen concentration and other acute phase proteins which have subjective influences on plasma viscosities. They did not show any variable influence and therefore maintained a relatively stable plasma viscosity (PV), whereas, in HbSS situation, it is known that the PV determinants are usually heightened due to the inflammatory responses in the patients and therefore, a raised value of PV is usually the case. The PFC values in this study significantly increased in HbSS when compared with HbAA and HbAS. This supported the observations in the PV obtained above and it is in line with the previous reports (Famodu *et al*., 1998; Ajayi *et al.*, 2005; Awodu *et al*., 2009).

We observed further in this study, the probable contributory roles of plasma and/or internal erythrocytic factors in whole blood viscosity. We replaced equal quantities of plasma with normal saline and recorded the relative erythrocyte viscosity values. There was no significant difference between HbAA and HbAS values, which confirmed the roles of both plasma and internal factors in whole blood viscosity. When HbSS values were compared with that of HbAA and HbAS, there was a significant decrease in the REV of HbSS ($P<0.05$, respectively). This shows that the concentration of hemoglobin within the erythrocyte of HbSS may be playing a determinant role in the internal viscosity of the red cells as well as the genotype of the hemoglobin and other membrane properties. In this case, the mean corpuscular hemoglobin concentration (MCHC) appeared to be lower than that of HbAS and HbAA, hence, the significant reduction in its viscosity. There is paucity of literature however on this observation and to our knowledge, no literature has attempted to explain this.

We conclude that, there are relatively stable whole blood viscosities in HbAA, HbAS and HbSS in their native states and that the increase in plasma viscosity of HbSS could be mostly determined by their plasma factors. It seems most likely that the genetic identity of the hemoglobin as well as its concentration are plausible factors in determining the cellular viscosity of erythrocytes

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