Effect of Diabetes Mellitus and Hypertension on Osmotic Fragility and Hemorheological Factors in Male Wistar Rats

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Abstract

Diabetes mellitus is a common risk factor for erythrocyte osmotic stress. This study was aimed at exploring the effect of streptozotocin (STZ)-induced diabetes mellitus and salt-induced hypertension on osmotic fragility and hemorheological variables in male Wistar rats. Thirty male rats were grouped into five groups of six animals each as follows: negative control (zero salt in diet); positive control (normal salt diet - 0.3% salt); high salt diet (8% salt) (HSD only); STZ induced diabetes and normal salt diet (STZ only); STZ induced diabetes and high salt diet (STZ + HSD). At the end of a 4 weeks period, hematological variables, osmotic fragility, rheology and cardiovascular responses were assessed. There was an increase (p<0.05) in the mean arterial pressure and heart rate of HSD, STZ and HSD + STZ groups indicating a salt induced hypertension. There was a decrease in the body weight of STZ and HSD +STZ groups. There was significant increase (p<0.05) in the haematocrit, platelets estimates and fibrinogen concentrations in the experimental groups when compared with the controls. The STZ and STZ + HSD groups showed a reduced clotting time which corresponded to the increased platelet estimates and fibrinogen concentration. The increase in haematocrit, platelet and plasma protein resulted in the increased blood viscosity and a decreased flow rate. The osmotic fragility test was also observed to be increased (p<0.05) in HSD, STZ only and STZ + HSD groups. Diabetes mellitus and hypertension increase the rate of hemolysis of erythrocyte, as well as increase blood viscosity.

Keywords: diabetes mellitus; hemorheology; high salt diet; hypertension; viscosity.

Abbreviations: BP – Blood pressure; DM – Diabetes mellitus; HR – Heart rate; HSD – High salt diet; NaCl – Sodium chloride; RPV – Relative plasma viscosity; SBP – Systolic blood pressure; STZ – Streptozotocin; WBV – Whole blood viscosity.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder in the endocrine system marked by abnormalities in insulin secretion and/or insulin action that leads to the progressive deterioration of glucose tolerance, which causes hyperglycemia (Granner, 2000). In postmodern times, DM has now become a trending public health problem which calls for serious care and concern (Harika et al., 2014). Various medical therapies are current being used in management of diabetes (Patel et al., 2010; Chaudhury et al., 2017; Palanisamy et al., 2018; Okafo et al., 2019). DM is one of the major risk factors for increased osmotic fragility. This is because hyperglycemia causes structural and functional changes in erythrocytes which can result in osmotic stress (Eze et al., 2017).

According to the American Heart Association, high salt consumption is a serious essential/primary hypertension cause and risk factor. Go reported that an average American adult consumes about 5400 mg of sodium per day which is about 130% increase more than the recommended 2300 mg of sodium per day (Go et al., 2013). This high salt intake per day is a major risk factor for essential hypertension. The rate of erythrocyte osmotic fragility increase in hypertensive subjects is higher than in normotensive subjects (Fasanmade, 1999). High salt intake which induces hypertension will cause an increase in the osmotic fragility of red blood corpuscle (Baskurt and Meiselman, 2003). High salt diets have been greatly linked to be a serious risk factor for cardiovascular diseases relating to high blood pressure, endothelial dysfunction, stroke, ventricular hypertrophy and fibrosis, arterial and ventricular stiffening, myocardial infarction, arrhythmias, and heart failure (Mohan and Campbell, 2009) while diabetes mellitus is a common risk factor for erythrocyte osmotic stress (Eze et al., 2017). This suggests that the

constituents of blood and its flow properties serve as a link between DM and hypertension.

Hemorheology is the study of the flow properties of blood and its elements (plasma and formed elements, including erythrocytes, white blood cells and platelets) Baskurt et al., 2007). Hemorheological parameters, such as hematocrit, plasma and whole blood viscosity, plasma proteins, erythrocyte deformability and aggregation are basic characteristics of blood flow (Rabai, 2013). There is growing evidence that the flow properties of blood are among the main factors of proper tissue perfusion, and shifts in these properties play significant roles in disease processes (Mohan et al., 2001). The viscosity of blood is directly proportional to the hemoconcentration and inversely proportional to the flow rate. This implies that factors which increase blood constituents and decrease plasma will elevate the blood viscosity which will decrease flow rate and alter tissue perfusion (Chang et al., 2017; Sloop et al., 2020). There are adequate evidences on diabetes that the elevated blood viscosity is a pathogenetic factor of diabetic microangiopathy, altering microcirculation and leading to insufficient tissue nutrition (Grigoleit et al., 1973; Cho et al., 2008). Alterations in tissue perfusion will result in microvascular and macrovascular complications (Cade et al., 2008).

Hence, this study provides an investigation into the rheological properties of blood in diabetic and hypertensive male Wistar rats.

MATERIALS AND METHODS

Experimental Design
A total of thirty male Wistar rats (120-150 g) were used for the experiment. They were acclimatized for two weeks prior to commencement of the experiment at the Central Animal House of the College of Medicine, University of Ibadan, Nigeria, and kept under standard laboratory conditions and fed standard rat pellets and water *ad libitum*. Animal handling was done in accordance to established guidelines by the National Institute of Health for care and use of laboratory animals. Ethical approval was given by the College of Medicine Ethics Committee (UI-ACUREC/19/0140).

The rats were grouped into five groups of six animals each as follows: negative control (zero salt in diet); positive control (normal salt diet - 0.3% salt); high salt diet (8% salt) (HSD only); STZ induced diabetes and normal salt diet (STZ only); STZ induced diabetes and high salt diet (STZ + HSD).

Preparation of Feed Rations
Three different rations were used for the study. The high salt diet was prepared by mixing 8g of table salt with 92 g of standard rat chow (Asiwe et al., 2020). HSD only and STZ +HSD groups were given this ration and water ad libitum. The normal salt diet was prepared by mixing

0.30 g of table salt with 99.70 g of standard rat chow which was given to the positive control group and water ad libitum. While the zero-salt diet was given to the negative control group.

Induction of Diabetes Mellitus
Diabetes was induced by single intraperitoneal injection of 60 mg/kg body weight dose of streptozotocin (STZ) dissolved in freshly prepared 0.1M cold citrate buffer of pH 4.5 into the animals according to the STZ-induced hyperglycemia in rats' model (Eze et al., 2017; Asiwe et al., 2020; Akbarzadeh et al., 2007). The experimental animals were fasted overnight (18 hours) prior to diabetes induction while allowed access to drinking water. Seventy- two hours after streptozotocin injection, a drop of blood was drawn from tail vein of the rats to measure their blood glucose level. Animals having fasting blood glucose levels $\geq$ 200 mg/dL were considered diabetic and used in the study.

Measurement of Blood Glucose Level
In order to ascertain the diabetic state of the animals, the fasting blood sugar level was measured every week after being fasted overnight using the ACCU-CHEK Active glucometer (Model GB06140695). The results obtained in mmol/L were converted to mg/dL by multiplying with 18 (conversion factor). Blood samples were collected from tail artery of the rats for evaluation of the blood glucose. Body weights of animals were also measured weekly in grams to estimate the effect of the induced diabetes and high salt diet on body composition. The weight was measured with a standard weighing scale.

Blood Pressure and Heart Rate
Blood Pressure and Heart Rate were done at the Small Animal Ward of the Veterinary Clinic University of Ibadan, Ibadan, Nigeria. Systolic Blood Pressure (SBP) was measured indirectly in a conscious and slightly restrained rat using the tail cuff plethysmography method (Kent Scientific, USA). Heart Rate (HR) tracings were observed and recorded as obtained during blood pressure (BP) measurement. For these measurements, rats were conditioned to the restraint (cone) and the warming chamber for about 20 minutes before the measurement. SBP and HR measurements were performed in a very quiet environment to avoid sound interference by the same investigator. There were two sensors to measure BP and Vascular Peripheral Resistance. After stabilization in the chamber, an acclimatization run was performed for 5 cycles which was immediately followed by the typical run involving 10 repetitions of the automated inflation-deflation cycle.

Examination of Blood Samples
Blood samples were collected by ocular puncture at the end of the fourth week and stored in heparinized bottles which were used to consider effect of high salt diet and

diabetes mellitus on hematology, osmotic fragility and rheology. Four animals were used for each group for analysis (n=4).

Rheological Analysis

Whole blood viscosity and relative plasma viscosity were estimated using the method described by Reid and Ugwu (1987), and the flow rate was calculated. Plasma fibrinogen concentration was estimated by clot weight method of Ingram (1952). Haematocrit was estimated using microhaematocrit reader.

Statistical Analysis

Data were analyzed with GraphPad Prism version 7.0 (GraphPad Software, San Diego, CA) and expressed as means ± SEM (standard error of mean). One-way ANOVA was used for comparisons, followed by post hoc Newman-Keuls Multiple Comparison test. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Osmotic Fragility

Figure 1 shows the rate of erythrocyte osmotic fragility (%). The percentage erythrocyte osmotic fragility decreased significantly with an increase in sodium chloride (NaCl) concentration. There was complete (100%) haemolysis at 0.0% of NaCl. And there were no significant changes in erythrocyte osmotic fragility when observed at 0.0% and 0.1% of NaCl concentration in all control and experimental groups when compared. However, significant ($P < 0.05$) changes in percentage erythrocyte fragility were recorded at 0.3%, 0.5%, 0.7% and 0.9% of NaCl concentrations, when the experimental groups were compared with the control groups. *a, *b, *c, and *d p<0.05 at 0.3%, 0.5%, 0.7% and 0.9% of NaCl concentrations.

Platelet Counts

Figure 2 shows the platelets estimates (mm^3) in diabetic and hypertensive male Wistar rats. There was significant increase when High salt diet + STZ groups were compared with the controls. $p < 0.05$ is significant when compared with negative control and positive control groups.

Fibrinogen Estimates

Figure 3 shows the fibrinogen estimates (mm^3) in diabetic and hypertensive male wistar rats. There was significant increase when other groups were compared with the controls. *p < 0.05 is significant when compared with negative control and positive control groups.

Hematocrit

Figure 4 shows the hematocrit (%) in diabetic and hypertensive male wistar rats. There was significant increase when other groups were compared with the controls. *p < 0.05 is significant when compared with negative control and positive control groups.

Whole Blood Viscosity

Figure 5 shows the whole blood viscosity (WBV) (mPa.s) in diabetic and hypertensive male wistar rats. From the values expressed there was significant increase between the negative control group and positive control group. There was significant increase when other groups were compared with the controls. **p < 0.05 is significant when the negative control group was compared with the positive control group; *p < 0.05 is significant when compared with negative control and positive control groups.

Relative Plasma Viscosity

Figure 6 shows the Relative Plasma Viscosity (RPV) (mPa.s) in diabetic and hypertensive male wistar rats. From the values expressed there was significant increase between the negative control group and positive control group. There was significant increase when other groups were compared with the controls. **p < 0.05 is significant when the negative control group was compared with the positive control group; *p < 0.05 is significant when compared with negative control and positive control groups.

Plasma Flow Rate

Figure 7 shows the plasma flow rate (cm/sec) in diabetic and hypertensive male wistar rats. There was significant increase between the negative control and positive control groups. There was significant increase when other groups were compared with the controls. **p < 0.05 is significant when the negative control group was compared with the positive control group; *p < 0.05 is significant when compared with negative control and positive control groups.
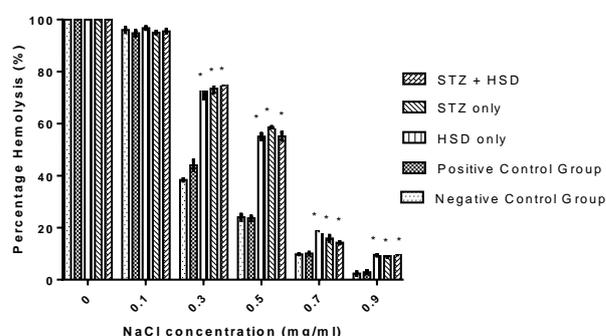
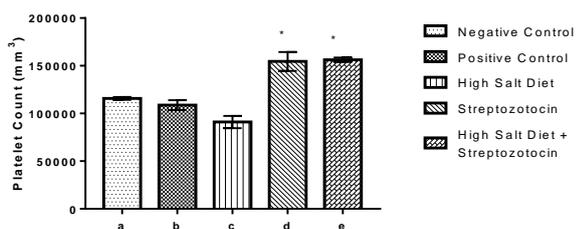**Figure 1.** Rate of erythrocyte osmotic fragility (%) in diabetic and hypertensive male Wistar rats.

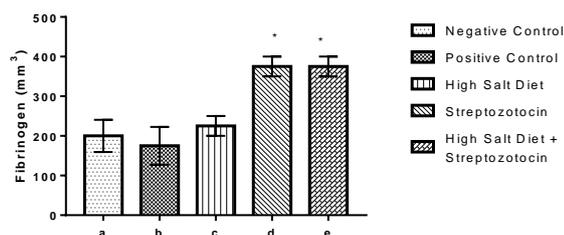**Figure 2.** Platelet count (mm^3) in diabetic and hypertensive male Wistar rats.

Figure 3. Fibrinogen estimates (mm^3) in diabetic and hypertensive male Wistar rats.
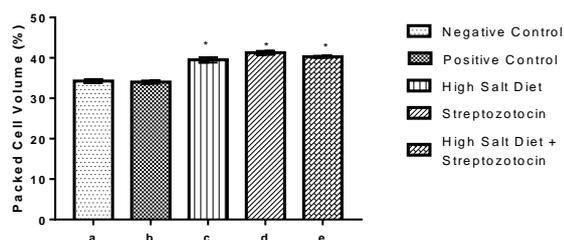

Figure 4. Hematocrit (%) in diabetic and hypertensive male Wistar rats.
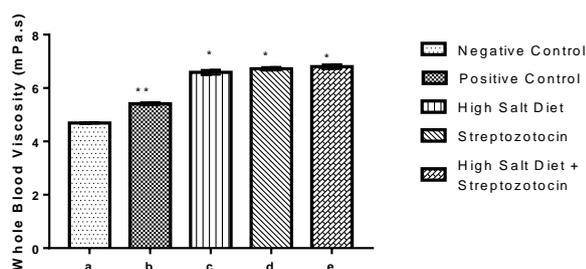

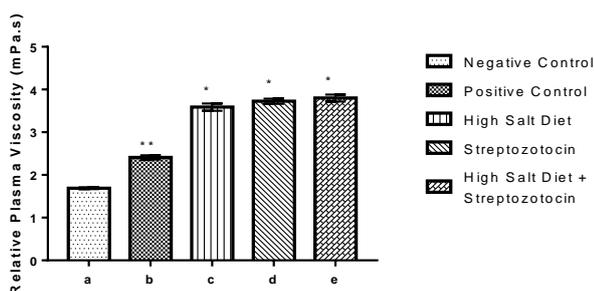**Figure 5.** Whole Blood Viscosity (mPa.s) in diabetic and hypertensive male Wistar rats.

Figure 6. Relative Plasma Viscosity (mPa.s) in diabetic and hypertensive male Wistar rats.
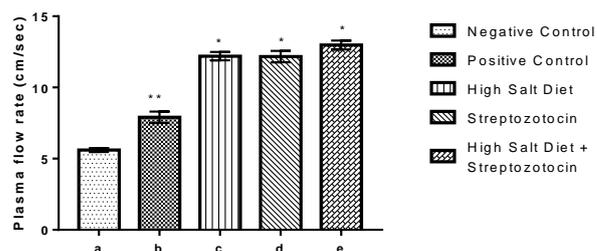

Figure 7. Plasma flow rate (cm/sec) in diabetic and hypertensive male Wistar rats.

In this study, the percentage erythrocyte osmotic fragility decreased significantly with increasing NaCl concentration. There was complete (100%) hemolysis at 0.0% and 0.1% of NaCl. No significant changes in erythrocyte osmotic fragility were observed at 0.0% and 0.1% of NaCl (distilled water) in both controls and experimental groups when compared. However, the results of the osmotic fragility showed a significant increase in the rate of erythrocyte osmotic fragility at 0.3%, 0.5%, 0.7% and 0.9% of NaCl concentrations in HSD only, STZ only and HSD +STZ groups, when compared with the control groups. This finding was in agreement with the works of other researchers (Shin et al., 2007; Megha and Sehyun, 2009; Eze et al, 2017) who showed that erythrocyte osmotic fragility was significantly increased in streptozotocin-induced diabetic animals. The increased rate of erythrocyte osmotic fragility in hypertensive subjects has also been reported by Fasanmade (1999). The implication of this finding suggests that STZ induced diabetes can alter the integrity of the red blood cell (RBC) membrane.

Erythrocyte osmotic fragility test gives an in-vitro measure of the tensile strength of erythrocyte membrane and it is an indirect method of evaluating lipid peroxidation in animals (Akande et al., 2014). It is also used to measure the tensile strength of erythrocytes and its ability to resist alterations in osmotic gradients and it has been discovered to be increased during oxidative stress (Aldrich et al., 2006; Adenkola et al., 2010). Hence, this suggests that the greater the erythrocyte osmotic fragility, the weaker the tensile strength of erythrocyte membrane (Ogundeji et al, 2013) and a resultant hemolysis of erythrocyte is observed. The mechanism for increased fragility of erythrocytes have been reported to be due to increased glycosylation of the erythrocyte membrane protein or/and alteration of the Na+/K+ ATPase on the erythrocyte membrane (Arun, 2013). The viability of an erythrocyte depends greatly on the maintenance of its membrane. Studies have shown that DM and hypertension can cause an increase in lipoperoxidative changes in the membranes of erythrocytes (Qujeq et al., 2005; Ahmed et al., 2006; Gauri and Vijaya, 2008) which account for the increase in the rate of osmotic fragility.

Hemorheology is the study of the flow properties of blood and its elements such as plasma with its dissolved

components and formed elements, which include erythrocytes, leucocytes and platelets (Baskurt et al., 2007). The flow properties of blood are among the main determinants of proper tissue perfusion, and alterations in these properties play vital roles in disease processes (Lowe et al., 1980). In this study, hemorheological factors were measured with focus on blood viscosity and flow rate. The viscosity of blood is determined by the hematocrit (Rabai, 2013; Cho et al., 2008), increase in plasma viscosity which is determined by plasma proteins (fibrinogen and globulin) and water (Mohan et al., 2001). From this study, there was a significant increase in the blood viscosity and plasma viscosity in the HSD only, STZ only and STZ+HSD groups when compared with the control groups. The observed increase in viscosity could be due to the significant increase in hematocrit, fibrinogen, and platelet estimates as recorded in STZ only and STZ+HSD groups. This finding was in accordance with the works of Reid and Memeh, and others (Reid and Memeh, 1988; Khan et al., 2005; Okomafe et al., 2017). Increased blood viscosity and the onset of diabetic angiopathy have been related to abnormal hematocrit, plasma viscosity, fibrinogen concentration, erythrocyte deformability and rouleaux formation (Winberger and Baskurt, 2007). The increase in hematocrit in the experimental groups appears to have contradicted the increased rate of erythrocyte osmotic fragility as reported earlier in this study. A possible mechanism to support this finding is linked to hemoconcentration. As the osmolarity of the blood increases due to increased glucose level, the capillary permeability increases, resulting in a decrease in plasma water and thus increasing hematocrit and subsequently the blood viscosity (Meiselman et al., 1967; Rizvi and Zaid, 2001). However, HSD only group had a significant increase in hematocrit only with a decrease in fibrinogen and platelet estimates when compared with other experimental groups. This increase in hematocrit could be the major reason behind the increase in blood viscosity (MacRury et al., 1988). Another interesting finding in this study was the significant increase in blood and plasma viscosity when the positive control group with normal salt intake was compared with the salt deficient negative control group. The possible mechanism underlying this claim is yet to be fully understood.

The flow rate of blood is determined by three main factors – vessel diameter, perfusion pressure and blood viscosity (Lowe et al., 1980). Although vessel diameter has been primarily linked with blood flow rate, in pathological conditions, the contributions of perfusion pressure and viscosity becomes greatly enhanced. It has been reported from studies that the viscosity of blood is directly proportional to the hemoconcentration and inversely proportional to the flow rate (Sloop et al., 2020). This implies that factors which increase blood constituents and decrease plasma water will elevate the viscosity of blood which will decrease flow rate and alter tissue perfusion (Chang et al., 2017; Sloop et al., 2020). From this study, it was also observed that there was a corresponding significant decrease in the flow rate of plasma across the experimental groups when compared with the control groups. Hence, the increase in blood viscosity could be a factor for the decreased flow rate of plasma. The alterations in the blood flow patterns in DM is produced by a combination of reduced erythrocyte deformability and increased erythrocyte aggregation due to variations in the plasma protein (McMillian et al., 1978). The changes in plasma proteins are linked with the development of glucose intolerance (McMillian, 1983). A decrease in flow rate can possibly result in circulatory insufficiency marked with poor tissue perfusion which in a long term can lead to the development of vascular complications as commonly observed in subjects with DM (Lowe et al., 1980). There was a significant decrease in plasma flow rate when the positive control group with normal salt intake (with higher blood and plasma viscosity) was compared with the salt deficient negative control group. This also verified the inverse relationship between blood viscosity and plasma flow rate.

CONCLUSION

From the findings of this study, it can be stated that diabetes mellitus and hypertension increase the rate of hemolysis of erythrocyte. There is also an increase in the hematocrit which significantly increases the blood viscosity alongside with plasma protein and decreased plasma water, thus, decreasing plasma flow rate. This could possibly result in circulatory insufficiency as well as a poor tissue perfusion which will lead to vascular complications.

Conflict of Interest: The authors declare that there are no conflicts of interest concerning the publication of this article.

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