



HAEMORHEOLOGICAL PARAMETERS OF APPARENTLY HEALTHY STUDENTS OF RIVERS STATE UNIVERSITY OF SCIENCE AND TECHNOLOGY, PORT HARCOURT.

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ABSTRACT

This study was aimed at providing baseline data on some haemorheological parameters in apparently healthy students of Rivers State University of Science and Technology, Port Harcourt, Nigeria. A total of 300 male and female students between the age of 18-45 years undergoing compulsory medical examination at the University's health centre participated in the study. Fasting blood samples were collected from the subjects and packed cell volume, erythrocyte sedimentation rate, platelets count, whole blood viscosity, plasma viscosity and fibrinogen levels were determined using standard methods. Results showed no significant difference ($p > 0.05$) in the means of the parameters between the age classes of the students. The mean of the PCV, Hb, WBV and PV were higher in the males than females while the means of the ESR, platelet count and fibrinogen were higher in the females than in the males. Significant difference ($p < 0.05$) in mean of PCV, Hb, platelet count, WBV, PV and fibrinogen and none significant difference ($p > 0.05$) in the mean of ESR between the males and the females was observed. Significant positive correlations were also observed between packed cell volume and haemoglobin ($r = 0.87$), while weak positive correlations were found between PCV and WBV ($r = 0.40$), PCV and PV ($r = 0.38$), Hb and WBV ($r = 0.31$), Hb and PV ($r = 0.33$), ESR and fibrinogen ($r = 0.35$), WBV and fibrinogen ($r = 0.35$) and PV and fibrinogen ($r = 0.38$). The present study further showed that sex variations in haemorheological parameters exist in apparently healthy students studied in Port Harcourt as observed in studies in other parts of the world.

KEYWORDS: Haemorrheology, packed cell volume, plasma viscosity, whole blood viscosity, fibrinogen.

INTRODUCTION

Haemorheology is the study of the flow properties of blood and its elements. The flow properties of blood have been suggested to be one of the main determinants of proper tissue perfusion and alteration in these properties play significant role in disease processes. The property of the fluidity and internal friction of blood is referred to as whole blood viscosity. Promotion and development of cardiovascular disease by all major risk markers are attributable to the mechanism of increased viscosity.^[1] Generally, changes in whole blood viscosity have been reported in several human cardiovascular diseases indicating that blood viscosity may be a major cardiovascular risk factor. Men have been shown to have higher blood viscosity than women, largely because of their higher haematocrit.

Plasma viscosity is the intrinsic flow resistance of plasma. Increased plasma viscosity and whole-blood viscosity are observed in primary hyperlipoproteinaemias as well as in secondary hyperlipoproteinaemias such as

diabetes mellitus and the nephrotic syndrome.^[1] Plasma viscosity is primarily dependent on the concentration of plasma proteins, especially fibrinogen and it is not affected by anaemia.^[2] According to^[3], elevated plasma fibrinogen levels may be regarded as an independent cardiovascular risk factor. Cardiovascular disease is associated with high fibrinogen and lipid fractions leading to an increase of both plasma and whole blood viscosity as well as raised aggregability of blood cells.^[4]

There is increased risk for CVD in response to arterial injury and vascular abnormality like atherosclerosis due to increased deposition of fibrin, cellular proliferation and fibrous scar formation.^[5,6] These are followed by decreased fibrinolytic activity, increased plasminogen activator inhibitor-1 activity (PAI-1) and decreased thrombus dissolution, which culminate in a pro-thrombotic state.^[6] Hyperglycemia and insulin resistance, both components of the metabolic syndrome (MS) enhances this state. However, the PAI-1 activity does decrease with insulin treatment.^[7,8] Hemorrheological

variations in plasma components lead to hyperviscosity that induces micro-vascular damage and facilitates occlusive events via erythrocyte rouleaux formation and platelet aggregation.^[8]

Recently, a series of studies have demonstrated that red blood cell distribution width can serve as a novel, independent predictor of prognosis in patients with cardiovascular diseases.^[9] There are few reports on the study of haemorheological parameters either as risk factors for CVD in Nigerian or as baseline study and there has been no such report from our center at the Rivers State University of Science and Technology, Nkpogu, Port Harcourt, Nigeria. This study therefore, sought to provide a baseline data and assess any possible sex variations in haemorheological parameters such as packed cell volume (PCV), erythrocyte sedimentation rate (ESR), platelets count (PC), whole blood viscosity (WBV), plasma viscosity (PV) and fibrinogen levels in apparently healthy students of Rivers State University of Science and Technology, Nkpogu, Port Harcourt, Nigeria.

MATERIALS AND METHODS

Subjects selection

A total of 300 apparently healthy young men and women who were students of the Rivers State University of Science and Technology, Nkpogu, Port Harcourt, Nigeria, undergoing compulsory medical examination at the University's health centre were recruited into this study

Ethical approval

Ethical approval was obtained from the Ethics Committee of Rivers State University of Science and Technology, Nkpogu, Port Harcourt, Nigeria, Health Services Department and informed consent was obtained from all participants that were involved in the study.

Inclusion criteria

Inclusion criteria were apparently healthy students admitted into the University and required to undergo the compulsory medical examination of the University. Exclusion criteria were pregnant women, subjects who admitted to bleeding or clotting disorders, including history of deep vein thrombosis, concurrent malignancy requiring cytotoxic chemotherapy or radiation therapy, subjects less than 18 years of age and subjects that declined from participating in the study.

Sample collection

The subject's vein was selected and the tourniquet was tied round the arm, the skin area was disinfected with methylated spirit and 10ml syringe and 21G needle size was used to draw blood from the ante cubital vein. About 4ml of blood was put into lithium heparin bottle, 3ml put into ethylenediamine tetra-acetic acid (EDTA) bottle while 3.8ml was put into sodium citrate bottle. All the bottles were labeled, capped and gently the blood was mixed with the anticoagulants.

METHODS

Estimation of haemoglobin concentration

This test was done using the modified azide methaemoglobin reaction method with Hb 201⁺ HemoCue Analyzer, model No. HemoCue AB, Sweden. The erythrocytes are haemolyzed to release the hemoglobin. The haemoglobin is converted to methaemoglobin and then combined with azide to form azidemethaemoglobin which was measured at two wavelengths in order to compensate for turbidity by the analyzer.

Determination of packed red blood cell volume (PCV)

The microhaematocrit method involving the use of the microhaematocrit centrifuge and microhaematocrit reader was used.^[10] Haematocrit (HCT) levels reflect the proportion of blood occupied by red blood cells (RBCs). When a well-mixed blood specimen in a capillary tube is centrifuged by the microhaematocrit centrifuge, the centrifuge provides a centrifugal force of 12000g and a 5 minutes centrifugation results in a constant Packed cell in the capillary tube referred to as packed cell volume (PCV).

Platelets count

Platelets count was done using the visual cell counts method by using the improved Neubauer counting chamber. Diluted blood was passed under a cover glass on a counting chamber and the chamber was placed in position on the microscope with the cover glass on it. The cover glass rests upon the two outer platforms of the chamber producing a clearance between itself and the rulings on the central platforms of the chamber. The clearance produced is referred to as the depth of the counting chamber.^[11]

Determination of erythrocyte sedimentation rate (ESR)

The method for measuring the ESR is based on that of Westergren, recommended by the International Council for Standardization in Haematology (ICSH). The erythrocyte sedimentation rate (ESR) measures the degree of red blood cells settling during a specified time of 1 hour. The red blood cells in diluted blood in an open-ended glass or plastic tube of 30cm length when mounted vertically on a stand, descend in the tube and displace an equal volume of plasma upward, which shows the downward progress of other settling blood elements.^[12]

Determination of whole blood viscosity (WBV)

The modified needle and syringe method of Reid and Ugwu^[13] was used. When anticoagulated blood is withdrawn into a syringe with the plunger, the blood cannot drop except pushed by the plunger. But in this method for the determination of blood viscosity, the plunger is removed for a flow without force. As the whole blood interacts with the wall of the syringe, it influences the flow and blood starts to drop. The relative

viscosity time was recorded compared to flow-time of distilled water at the same temperature in seconds.

Determination of plasma viscosity

Plasma Viscosity (PV) was carried out by a modification of the method of Reid and Ugwu.^[13] For the plasma viscosity, a part of the whole blood was centrifuged in a stoppered sterile clean bottle to obtain clean and clear plasma. It was centrifuged for 5 mins at 3000g. The result was calculated in the same way as that of the whole blood viscosity.

Plasma fibrinogen estimation

Fibrinogen was estimated using the modified method of Clauss.^[14] The modified Clauss method involves the use of the fibrinogen reagent kit, manufactured by Technoclone, Vienna, Austria. The determination of fibrinogen with thrombin clotting time is based on the method originally described by Clauss; in the presence of an excess of thrombin, fibrinogen is transformed into fibrin and clot formation time is inversely proportional to the concentration of fibrinogen in the sample plasma.

Statistical analysis

The results were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version

21. Data were expressed as mean \pm SD. Analysis of variance (ANOVA) was used to compare mean differences among groups, Pearson correlation was used to investigate linear correlation amongst the parameters whilst student t-test was used to compare the differences between two groups. Values were considered significant at $p < 0.05$.

RESULTS

A total of 300 apparently healthy young men and women who were students of the Rivers State University of Science and Technology, Nkpogu, Port Harcourt, Nigeria, undergoing compulsory medical examination at the University's health centre were recruited into this study for the purpose of assessing their baseline haemorrhological status. Of this number, 169 (56.3%) were males while the remaining 131 (43.7%) were females. The subjects were mainly young men and women and their distribution into age brackets and their percentages showed that 25.7% and 21.3% were between 18-24 and 25-29 years respectively while 21.0% and 20.0% were within 35-39 and 40-45 years respectively. Only 12.0% were in the 30-34 years (table 1).

Table 1: Demographic characteristics of the study population (males and females combined)

Age groups (years)	Number (n)	Percentage (%)
18 – 24	77	25.7
25 – 29	64	21.3
30 – 34	36	12.0
35 – 39	63	21.0
40-45	60	20.0
Total	300	100.0

Table 2: Comparison of means \pm SD of haemorrhological parameters according to age group (males and females combined)

Age-groups (years) (n)	PCV (%)	Hb (g/dl)	ESR (mm/hr)	PL ($\times 10^9/L$)	WBV (mpas)	PV (mpas)	Fibr (mg/L)
18-24 (77)	40.2 \pm 2.2	13.4 \pm 2.0	13.2 \pm 5.6	184.1 \pm 22.1	4.15 \pm 0.59	1.26 \pm 0.02	205.93 \pm 23.4
25-29 (64)	39.3 \pm 3.2	13.12 \pm 2.3	14.4 \pm 4.5	193.8 \pm 11.5	3.17 \pm 0.42	1.76 \pm 0.08	233.6 \pm 33.6
30-34 (36)	40.4 \pm 4.4	13.4 \pm 3.5	11.6 \pm 3.6	185.6 \pm 30.3	4.02 \pm 0.31	1.23 \pm 0.04	222.8 \pm 45.6
35-39 (63)	39.6 \pm 1.6	13.0 \pm 2.6	14.0 \pm 6.7	191.2 \pm 30.7	4.20 \pm 0.44	2.01 \pm 0.07	252.0 \pm 22.9
40-45 (60)	39.1 \pm 2.1	13.0 \pm 2.2	13.0 \pm 2.6	181.6 \pm 23.8	4.02 \pm 0.34	1.96 \pm 0.05	262.1 \pm 44.7
F value	0.907	1.099	1.066	0.740	1.809	1.494	5.783
P-value	ns	ns	ns	ns	ns	ns	p<0.05

Note: PCV= packed cell volume, Hb = haemoglobin, ESR= erythrocyte sedimentation rate, Pl= platelets, WBV=whole blood viscosity, PV= plasma viscosity and Fibr = fibrinogen, ns= not significant.

The mean and standard deviations of the packed cell volume (PCV), haemoglobin concentration (Hb), erythrocyte sedimentation rate (ESR), platelet count (PL), whole blood viscosity (WBV), plasma viscosity (PV) and fibrinogen (Fibr) in the subjects (combined) classified by age range is shown in table 2. There was no significant difference ($p > 0.05$) in the means of the parameters between the age classes. The means and standard deviations of the parameters in apparently

healthy male and female subjects is shown in table 3. The mean of the PCV, Hb, WBV and PV are higher in the males than females while the means of the ESR, platelet count and fibrinogen is higher in the females than in the males. Analysis of the data between the males and females using student t-tests showed significant difference ($p < 0.05$) in mean of PCV, Hb, platelet count, WBV, PV and fibrinogen while no significant difference ($p > 0.05$) was noticed in the means of ESR between the

males and the females respectively. Table 4 demonstrates the Pearson correlation between the parameters. Significant positive correlations were observed between packed cell volume and haemoglobin ($r=0.87$), while weak positive correlations was found between PCV and WBV ($r=0.40$), PCV and PV ($r=0.38$), Hb and WBV ($r=0.31$), Hb and PV ($r=0.33$), ESR and fibrinogen ($r=0.35$), WBV and fibrinogen ($r=0.35$) and PV and fibrinogen ($r=0.38$).

DISCUSSION

The results of the present study clearly indicate that sex variations occur in some haemorheological parameters in the apparently healthy populations studied. Haemoglobin concentration, haematocrit and specific gravity of whole blood have been reported to be significantly lower in healthy Nigerian females compared to males.^[15] The higher and significant ($p<0.05$, $F=32.54$) erythrocyte sedimentation rate, the lower but significant ($p<0.05$, $F=44.21$) haematocrit and haemoglobin ($p<0.05$, $F=29.55$) found in the females compared to males is consistent with earlier reports in this regard.^[16,17,18] Erythrocyte sedimentation rate (ESR) is called an acute phase reactant test because it reacts to acute conditions in the body such as infection and trauma. The rate of increase follows a rise in temperature and white blood cells count, peaks after several days and usually lasts longer than the elevated temperature or white blood cell count.^[19] In this study, we did not consider the relationship between body temperature and white blood cell count on the erythrocyte sedimentation rate. The ESR values in women may also be attributed to their low haematocrit or haemoglobin concentration occasioned by menstrual blood loss. In anaemia, with the haematocrit reduced, the velocity of the upward flow of plasma is altered so that red blood cell aggregates fall faster.^[20]

In addition, we also report that females in this study showed significant lower whole blood relative viscosity ($p<0.05$, $F=7.23$) and plasma viscosity which was also statistically significant ($p<0.05$, $F=12.53$). Blood viscosity is an important determinant of rate of blood flow and the greater the viscosity, the less the flow in a vessel, if all other factors are constant. Furthermore, the viscosity of normal blood is about three times as great as that of water. What makes blood so viscous is mainly the large number of suspended red cells in the blood, each of which exerts frictional tray against adjacent cells and

against the wall of the blood vessel.^[21] In this study, males recorded significantly higher mean value of relative plasma viscosity compared to the females ($p<0.05$, $F=12.53$). Thus, males may be at a higher risk of developing rheological abnormalities such as atherosclerosis, cardiovascular diseases and stroke in this population than females.

Blood viscosity is affected by the alteration in the concentration of biochemical factors in plasma. For instance, red blood cell aggregability which is induced by fibrinogen, is well documented. Similarly, red blood cell filterability/deformability and rigidity are not only influenced by external and internal constituents of red blood cells but to a larger extent by the membrane characteristics.^[22] We have selected hemoglobin along with haematocrit as major factors contributing to blood viscosity. The many studies that describe the influence of percentage hematocrit upon blood viscosity are essentially similar in their conclusions as to the degree of effect red cell concentrations have upon viscosity values.^[23,24] Our results showed a strong correlation between PCV and haemoglobin ($r=0.87$), moderate correlation between PCV and WBV ($r=0.40$), haemoglobin and PV and PCV and PV ($r=0.38$) which is in accordance with results of previous studies.^[22]

It is fairly established that sex variations occur in the various haemorheological parameters in healthy Africans.^[15,25,26] For example, fibrinogen concentration has been reported to be consistently (though not always significantly) higher in the female African compared to the African male. In this study, level of fibrinogen was observed to be significantly ($p<0.05$, $F=10.72$) higher in the females than in the males. Higher fibrinogen concentration has been reported to apparently account for elevated erythrocyte sedimentation rates usually seen in African females, an observation which was also seen in this study.^[15,26] Perhaps, these variations in both haemorheological and haematological parameters might possibly be responsible for the sex variations in blood and plasma viscosity in health and disease.

In conclusion, the result of the present study confirms that sex variations in haemorheological parameters exist in apparently healthy students studied at the Rivers State University of Science and Technology, Port Harcourt as observed in studies in other parts of the world.

Table 3: Comparison of means \pm SD of haemorheological parameters in subjects according to gender

Sex	PCV (%)	Hb (g/dl)	ESR (mm/hr)	PL ($\times 10^9/L$)	WBV (secs)	PV (secs)	Fibr (mg/L)
Male n=169	41.1 \pm 3.5	13.6 \pm 1.2	11.4 \pm 6.2	183.1 \pm 22.4	4.25 \pm 0.33	1.98 \pm 0.54	221.9 \pm 56.77
Female n=131	37.9 \pm 4.1	12.6 \pm 2.5	15.9 \pm 5.7	193.0 \pm 18.55	2.83 \pm 0.87	1.76 \pm 0.87	251.3 \pm 77.98
F-value	44.210	29.546	32.54	3.332	7.225	12.534	10.720
P-value	$p<0.05$	$p<0.05$	$p<0.05$	ns	$p<0.05$	$p<0.05$	$p<0.05$

Note: PCV= packed cell volume, Hb = haemoglobin, ESR= erythrocyte sedimentation rate, Pl= platelets, WBV=whole blood viscosity, PV= plasma viscosity and Fibr = fibrinogen, ns = not significant.

Table 4: Pearson Correlation of the parameters investigated

	PCV (%)	Hb (g/dl)	ESR (mm/hr)	Pl ($\times 10^9$)	WBV (mpa)	PV (mpa)	Fibr (mg/L)
PCV (%)	1.00						
Hb (g/dl)	0.87**	1.00					
ESR (mm/hr)	-4.04	-0.38	1.00				
Pl ($\times 10^9$)	-0.24	-0.20	0.19	1.00			
WBV (mpa)	0.36*	0.31*	-0.27	-0.24	1.00		
PV (mpa)	0.38*	0.33*	-0.33	-0.23	0.17	1.00	
Fibr (mg/L)	-0.100	-0.04	0.35	0.04	0.35	0.38*	1.00

** strong correlation

*weak correlation

Note: PCV= packed cell volume, Hb = haemoglobin, ESR= erythrocyte sedimentation rate, Pl= platelets, WBV=whole blood viscosity, PV= plasma viscosity and Fibr = fibrinogen.

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