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Age and gender specific variations of selected hemorheological variables in humans

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Abstract

Over the last decades, investigations have shown that the physical and mechanical damage of red blood corpuscles may significantly affect the fluency and diffusion state of blood flow, alongside internal viscosity and fibrinolytic activities of the cardiovascular system that houses it. Current study was thus envisioned to investigate gender and age differences in selected hemo-rheological parameters [pH, Whole Blood Viscosity (WV), Plasma Viscosity (PV), Plasma Fibrinogen Concentration (PFC), Packed Cell Volume (PCV) and Euglobinlysis time (ELT)] of adult humans. One hundred and forty (140) participants of seventy two (72) males and sixty eight (68) female subjects were ethically approached from the staff and students' community of the University of Benin, Edo state, Nigeria. Participants' health status was carefully obtained by means of history taking and questionnaire, following which individuals were grouped into two based on gender. For each gender, subjects were sub-grouped into the ages of 31-40 years, 41-50 years, 51-60 years, and 61-70 years based on reported incidences (by age) of hemo-rheological anomalies. For each subject, blood samples were obtained and assayed for aforementioned hemo-rheological changes. Statistical comparison (using the one way analysis of variance - ANOVA) was then conducted on collected samples for comparison of differences in mean between groups. From obtained result, study found a statistically insignificant difference in all but WV across gender and age lines, with slight increase in all other parameters. However, none of the parameters was significantly different across age groups, suggestive that the parameters do not differ across age group in the male participants.

Keywords: Hemo-rheology, Age, Gender.

INTRODUCTION

Rheology, the science and study of the material flow and movements through vessels (Zinngg and Shepley, 1970) [1] also concerns with the distortion of genetic stores under the influence of pressure (Brun *et al.,* 2007) [2]. In medical terms, rheology considers blood flow through the vascular system, in what is today known as hemorheology.

In specific terms, the rate of blood flow through the body's circulatory system has been shown to be dependent on heart pressure, frictional gradients amid endothelial cells, peripheral vessels and the blood itself. These factors apparently produce shear-stress on endothelial walls due to the innate forces of viscosity of vascular endothelium and the blood within the vessel itself (Brun *et al*., 2007) [2]. In a comparative study on fibrinogen levels and plasma viscosity of hypertensives and normotensives, a statistically significant increase in body weight, fibrinogen levels relative plasma viscosity and body mass index was reported for hypertensive diabetics than normotensives who were diabetic (Taj *et al*., 2005) [3].

By increasing the density of the plasma protein, studies have also shown an increase in plasma viscosity as well. Albeit, dissimilar parameters had different changes on viscosity including age and gender. Though there is a huge relationship between plasma globulin, viscosity and fibrinogen levels, albumin level showed less effect viscosity, but may be age and gender dependent. Incremental levels of viscosity of plasma is umpteen time, a key characteristics of the rheological ailments.

Recently, a significantly higher level of fibrinogen was found in relation to plasma viscosity among hypertensive and normotensive Nigerians; following comparison (Chien, 1977) [6]. These observations are traceable to an elevated level of fibrinogen seen in hypertensives; even though gender variations may have not been fully reported. Such report may be vital, considering that gender differences in rheological variables is reportedly elevated in normotensive Africans (Ingram, 1961; Dacie and Lewis, 1991; Dapper, 2002) [7,8]. Thus, gender variations in the pattern of rheological fluctuations at various age groups need to be elucidated.

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Ighoroje and Dapper did a work on the hemorheological parameters of hypertensives compared with normotensives. They reported a significant decrease in systolic, mean arterial pressures, haematocrit counts and relative blood viscosity; while reporting a significant increase in erythrocyte sedimentation rates (ESR) of female participants as against male subjects. Ighoroje and Dapper also reported that no relationship exists between hemorheological variables and blood pressures across gender lines. In hypertensives, relative blood viscosity was observed to have significantly increased in females than the male folks, while male hypertensives showed a significant, positive relationship for ESR and blood pressure variables; as well as in relative plasma viscosity. A negative correlation was also found for haematocrit, systolic and mean arterial pressures. Hypertensives female had positive correlations in their plasma viscosity, blood pressure variables as well as in relative viscosity and systolic pressure.

Aim of Study

This study aimed at investigating the gender and aged based differences in hemorheological in adult humans. Specifically, study compared average differences of selected hemorheological variables in male and female hypertensives. Study also determined the changes in selected hemorheological variables for different adult ages; of 31-40 years, 41-50 years, 51-60 years, and 61-70 years.

MATERIALS AND METHOD

Humans:

A total of one hundred and forty (140) participants, comprising of seventy two (72) males and sixty eight (68) female subjects were ethically recruited from the staff and students community of the University of Benin, Benin City, Edo State, Nigeria.

Reagents and Anti-Coagulants

1. 10g of dipotassium salt dissolved in 100ml of distilled water. 3.8% sodium citrate dissolved in 100ml of distilled water.

2. 0.0025M $CaCl_2$ (2.77g of anhydrous grade $Cacl_2$ reagent dissolved in clean distilled water, and made up to 1L, this solution was refrigerated at 4°C).

3. Dilute acetic acid (13.5ml of 1% v/v into 1000ml distilled water thoroughly mixed and stored in the fridge).

4. Borate buffer (9.0g NaCl plus 1.0g of sodium borate in 1L of volumetric flask filled with distilled water).

Questionnaire

Using a carefully structures questionnaire, participants' health status was first ascertained, and their base line data (weight, Blood Pressure and BMI) status obtained. The questionnaire was structured to be void of unnecessary questions and/or ambiguity.

Ethical Clearance:

Ethical clearance was obtained from the bio-research and ethics committee of the College of Medical Sciences, University of Benin, Benin City, Edo State, Nigeria.

Informed Consent:

Participants' written and oral consent was also obtained before investigation.

Sample and Sampling Technique

Using the simple random sampling technique, a convenient, cross-sectional sample size of One hundred and forty (140) participants were drawn from within the staff and students community of the University of Benin, Benin City, Edo State, Nigeria. Obtained samples (participants) were then grouped into two based on gender. For each gender, subjects were sub-grouped into the ages of 31-40 years, 41-50 years, 51-60 years, and 61-70 years based on reported incidences (by age) of hemo-rheological variations.

Selection Criteria

Subjects with known history of endocrine, metabolic, cardiovascular and any other medical disorders (as observed from questionnaire) were excluded from the study. Selection of subjects into groups was by gender, with ages groups of 31-40 years, 41-50 years, 51-60 years, and 61-70 years; based on reported incidences (by age) of hemo-rheological changes.

Blood Sample Collection

Soon after the obtaining participants' anthropometric parameters (with assistance from a trained phlebotomist), Blood samples were then collected form subjects' antecubital vein (at sitting position) in the cubital fossa. 9.5ml of blood was then taken from each person with minimum stasis using a 10ml syringe. Obtained blood was then dislodged into two 5ml specimen containers. 5ml of blood was added into a container containing 0.2ml of 10% ethylene diamine tetra acetic acid (EDTA) and immediately mixed. Next, 4.5ml of blood was added into another container containing 0.5ml of 3.8% sodium citrate and mixed immediately, then, kept in an ice filled container. Samples were immediately assayed for Whole Blood Viscosity (WV), Plasma viscosity (PV), Plasma Fibrinogen Concentration (PFC), Packed Cell Volume (PCV) and EuglobinLysis Time (ELT).

Determination of Whole Blood and Plasma Viscosity

(Method of Reid and Ugwu, 1987**)**

In principle, the test is based on the comparison of the flow rate of the whole blood/plasma and distilled water under equal pressure and constant temperature through capillary tube of equal bore and length, the result as expressed as whole blood/ plasma relative to that of water.

Instrument (capillary tub) was calibrated at room temperature against a controlled chamber used by Reid and Ugwu, (1987). The measurement of the flow rate of clean distilled water at room temperature was made by running 20 successive runs and the average was taken. The fluid (plasma or whole blood mixed with EDTA) to be tested was drawn up, cautiously excluding air bubbles in the vertical syringe until the plunger passed the 1.0 mark. The plunger was then completely withdrawn and immediately the lower meniscus fell to the 1.0 mark, a stopwatch was then started. The time required for 1.0ml of the fluid to drain down the syringe was taken. This was repeated twice on each sample and the mean flow rate was calculated. The whole blood relative viscosity and relative plasma viscosity was then calculated with the formula;

Whole blood relative viscosity / relative plasma viscosity = Test flow rate

Determination of Plasma Fibrinogen Concentration

(Ingram's clot weight method, 1961**)**

In principle, when calcium chloride is added to citrate plasma, this triggers off the intrinsic pathway of coagulation resulting in the

formation of fibrin cloth, which can be collected, weighed and by calculating the weight of fibrinogen is obtained.

To achieve this, 1ml of citrated plasma was pipetted into labeled test tube in water bath at 37°C. Then 1ml of pre warmed 0.025M $Cacl_2$ was added into the test-tube and mixed. The mixture is left in the water bath for 30 minutes with an applicator stick dipped into the test tube so that clot can be wound around it. When all the adherent fibrin are removed from the applicator stick after washing 3 times with distilled water and then blot dry carefully with whittman filter paper. Then adherent fibrin is carefully removed from the applicator stick into clean-labelled Petri dishes and kept in an hot air oven to dry.

Calculation:

$$\frac{\text{Dry weight x10/100ml}}{\text{Volume of plasma}}$$

Euglobin Lysis Time

(Methodology by Cecil Hougie 1967)

4½ ml of blood was collected plus 0.5ml of 1M sodium citrate into chilled 10ml centrifuge tube. The tube was placed in a refrigerator ice chambers at temp -20 to -10°C for 2 minutes, then it was kept in ice chambers for 5minutes, and centrifuged at 2000rpm for 10 minutes. The Plasma was separated and kept in an ice chambers, plasma was then duplicated. 0.5ml of plasma was mixed with 9.5ml of dilute acetic acid, tube containing plasma and dilute acetic acid was refrigerated at 4°C for 30 minutes and afterward centrifuged at 2000rpm for 10 minutes. Precipitant was formed, supernatant fluid was discarded, then 0.5ml of borate buffer was added to the precipitant and dissolved with a glass rod, the solution was placed in a water bath at 37°C for 2 minutes. 1.5ml of 0.025M $Cacl_2$ was added to solution, and then the solution clotted. A timer was started immediately the clot was formed. The tubes were placed in warm water bath at 37°C. The clot was observed at a regular interval of 2 minutes for lysis. At the point where the clot was completely lysed, the timer was stopped and the reading on the timer was noted.

Calculation:

ELT= interval between clotting and complete lysis.

Fibrinolytic Activity (FA)= $10^6 / ELT^2$, ELT is in minutes.

Statistical Analysis

Obtained data were analysed using appropriate descriptive statistics (mean, standard deviation). Were applicable, Student t-test and/or Analysis of variance (ANOVA) were used to obtain differences in mean between groups with $p < 0.05$ taken as statistically significant.

RESULTS

Table 1: Comparative Changes in Hemorheological Variables of Male and Femal Participants

Hemorheological Parameters	Male	Female	p-value
SBP(mmHg)	141.91±1.89	121.18±1.81	p < 0.05
DBP(mmHg	92.2±1.34	78.42±1.41	p < 0.05
MAP(mmHg)	106.03±1.42	92.78±1.05	p < 0.05
PR(b/min)	83.66±1.31	92.7±1.42	p < 0.05
WEIGHT(kg)	83.17±1.39	73.55±0.68	p < 0.05
HEIGHT(m)	1.7±0.02	72.15±1.66	p > 0.05
BMI(kg/m2)	28.26±0.38	1.66±0.01	p < 0.05
SG	1.02±0	26.21±0.5	p > 0.05
PCV(%)	41.57±0.37	6±0.04	p > 0.05
RWBV	7.51±0.29	1.02±0	p < 0.05
RPV	2.64±0.04	37.33±0.52	p < 0.05
FA	10.69±0.73	26.37±2.26	p < 0.05
PFC(mg)	70.89±3.58	55.56±5.79	p < 0.05

Above table shows a clear significance in the SBP, DBP, PR, MAP and the BMI between the hypertensive and normotensive adult males. In the hemorheological parameters RWBV, RPV and PFC were significantly higher in hypertensive male subjects, although PCV was also higher among hypertensive male subjects but it was not significant. FA was significantly higher in male normotensives compared to male hypertensives. Data is expressed as Mean ± SD. p < 0.05 = significant, p > 0.05 = Insignificant.

Table 2: Haematological Changes in Male Subjects across Age Groups

Parameters	31 -40 YRS	41 -50 YRS	51 -60 YRS	61 -70 YRS	p-value
SBP(mmHg)	149 [a]±3.67	141.1 [b]±3.59	146.2 [a] ±3.62	140.9 [b] ±2.83	p < 0.05
DBP(mmHg	91.2±1.02	91.4±3.23	92.9±2.16	94.8±2.44	p > 0.05
MAP(mmHg)	107.6 ±1.75	108.56±3.53	107.4 ±2.46	101.6±2.3	p > 0.05
PR(b/min)	81.2±3.02	81.5 ±2.76	84.5±2.37	86.2±2.39	p > 0.05
WEIGHT(kg)	84.2±4.53	83.9±2.18	83.7±2.43	81.4±3.11	p > 0.05
HEIGHT(m)	1.7±0.02	1.71±0.01	1.72±0.01	1.66±0.07	p > 0.05

BMI(kg/m2)	28.8±1.07	28.7±0.56	28.3±0.63	27.5±0.93	p > 0.05
SG	1.02 [a] ±0	1.02 [a] ±0	1.01 [b] ±0	1.02 [b] ±0	p < 0.05
PCV(%)	41.8±1.11	41.4±0.69	41.6±0.69	41.6±0.75	p > 0.05
WBRV	7.12±0.6	8.25±0.82	6.76±0.42	7.72±0.26	p > 0.05
RPV	2.66±0.11	2.74±0.09	2.58±0.09	2.61±0.04	p > 0.05
FA	11.39±2.21	11.69±0.89	8.54±1.07	11.49±1.81	p > 0.05
PFC(mg)	78.6±7.3	67.8±7.11	71.6±6.75	69.4±7.54	p > 0.05

p < 0.05- significant, p > 0.05- Insignificant. Above table shows that among male hypertensive subjects in different age groups there was no significant difference in all parameters apart from SBP across the age groups. This reflects that the parameters do not differ across age group in the hypertensive male.

Table 3: Haematological Changes in Female Subjects across Age Groups

Parameters	31 -40 YRS	41 -50 YRS	51 -60 YRS	61 -70 YRS	p-value
SBP(mmHg)	154.25±6.75	145.3±5.77	141.27±3.8	141.1±5.16	P>0.05
DBP(mmHg	98 [a]±7.12	94.7[b]±4.43	90.27 [b]±2.26	91.7 [b]±2.77	*P<0.05
MAP(mmHg)	116.5 [a]±6.95	113.6 [b]±3.98	102.82 [b]±3.62	100.3 [b]±3.88	*P<0.05
PR(b/min)	79.75±1.89	80.3±1.55	84.73±2.23	85±1.76	P>0.05
WEIGHT(kg)	79.5±2.18	76.6±2.14	76.91±2.32	79.2±2.13	P>0.05
HEIGHT(m)	1.66±0.02	1.64±0.01	1.65±0.01	1.68±0.01	P>0.05
BMI(kg/m2)	28.75±1.32	28.3±0.63	28.09±0.79	28.2±0.81	P>0.05
PH	6±0	5.8±0.13	6±0	6±0.15	P>0.05
SG	1.02±0	1.01±0	1.01±0	1.01±0	P>0.05
PCV(%)	40.5±1.66	37.9±0.6	37.55±0.49	38.5±1.14	P>0.05
WBRV	5.85 [b]±0.52	7.56 [a]±0.49	6.37 [b]±0.39	7.56 [a]±0.41	*P<0.05
RPV	2.51±0.23	2.47±0.16	2.62±0.11	2.66±0.14	P>0.05
FA	9.73±2.18	7.8±0.78	9.6±1.48	8.49±0.8	P>0.05
PFC(mg)	70.5±10.74	55.2±7.14	79.64±7.73	70.6±8.57	P>0.05

Above table shows a significant difference in DBP, MAP and WBRV across the age groups of Hypertensive female. The DBP, MAP was significantly higher in younger females than the older females. The younger females relatively had a higher significance in terms of SBP. WBRV was also higher in hypertensive females in the age groups 41-50 and 61-70. However there was no significance in other parameters across the age groups.

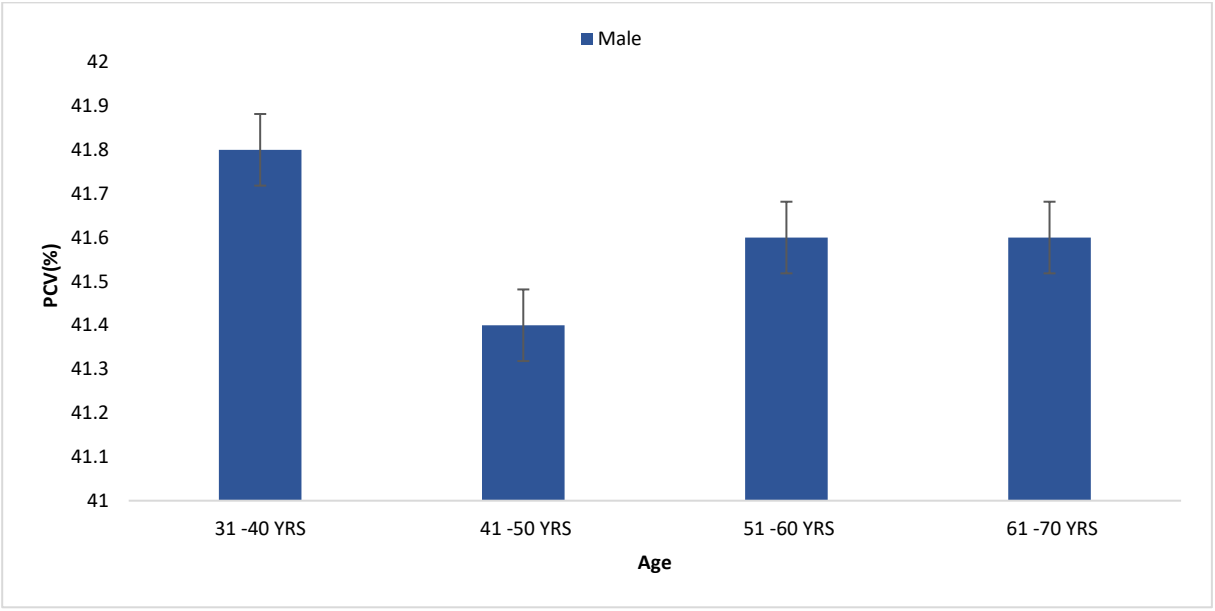

Figure 1: Age based Comparison of Packed Cell Volume in Male subjects

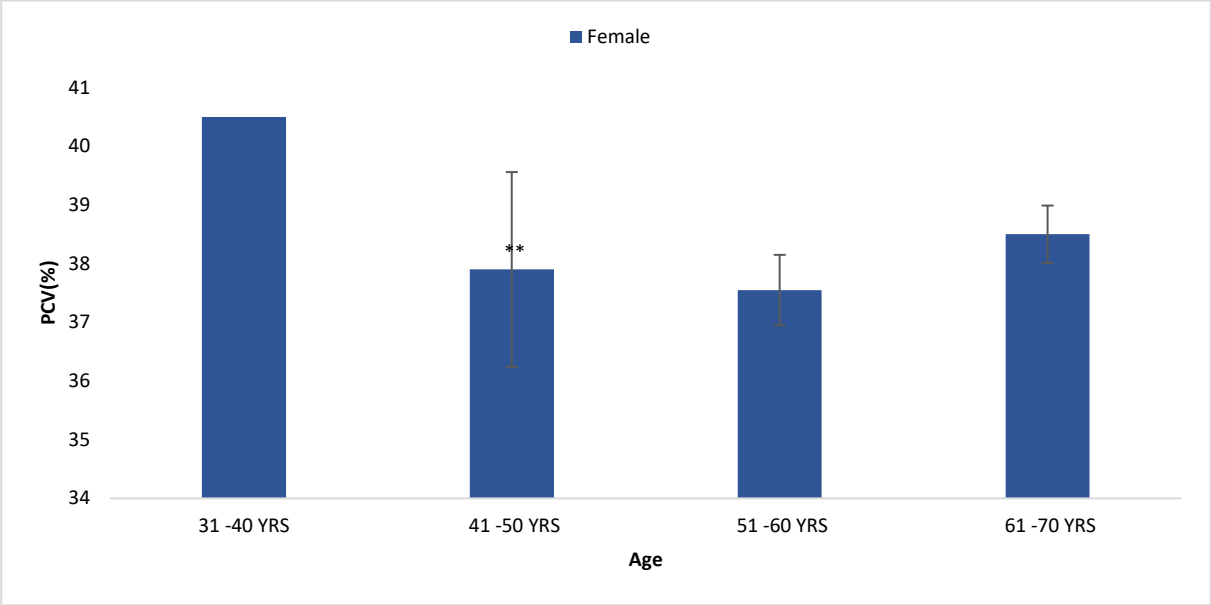

Figure 2: Age based Comparison of Packed Cell Volume in Female subjects

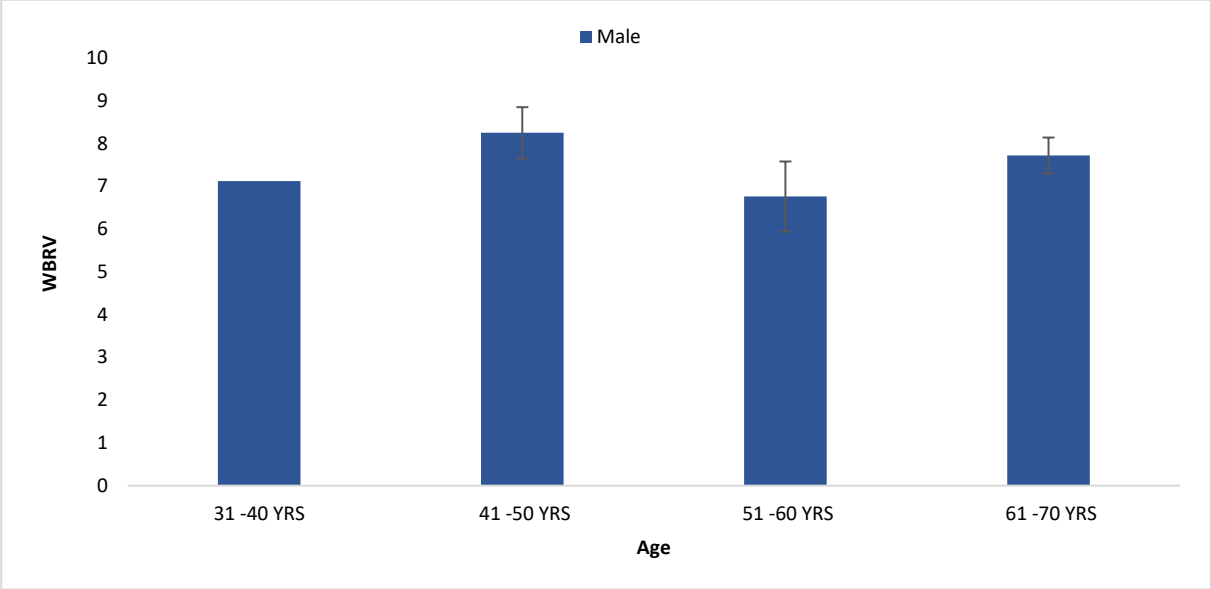

Figure 3: Age based Comparison of Whole Blood Relative Volume in Male subjects

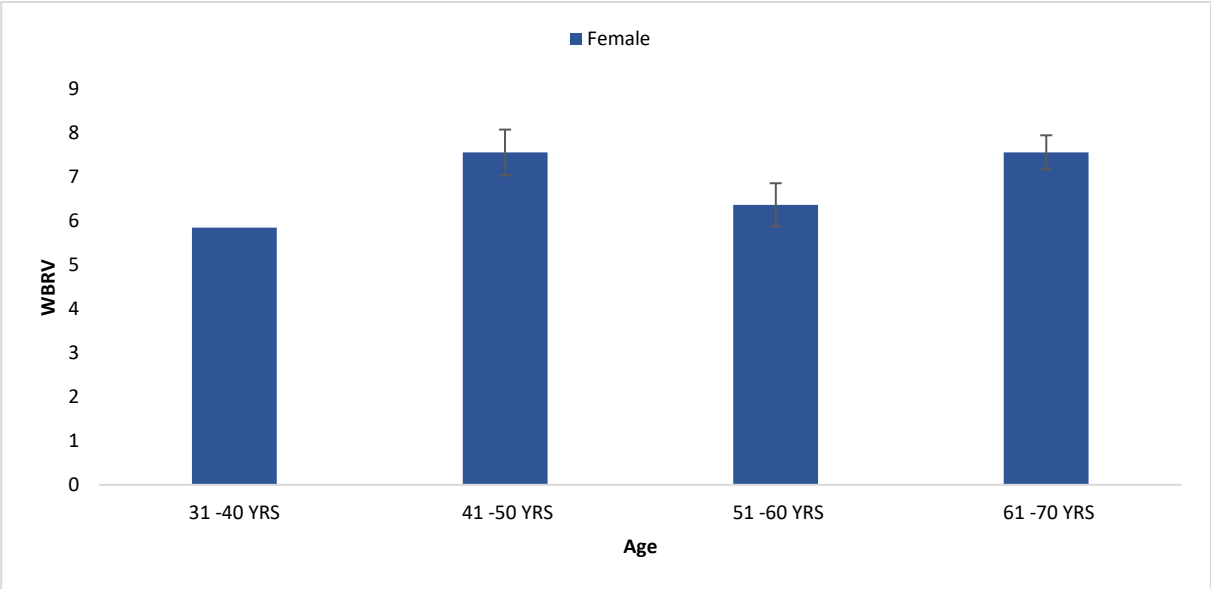

Figure 4: Age based Comparison of Whole Blood Relative Volume in Female subjects

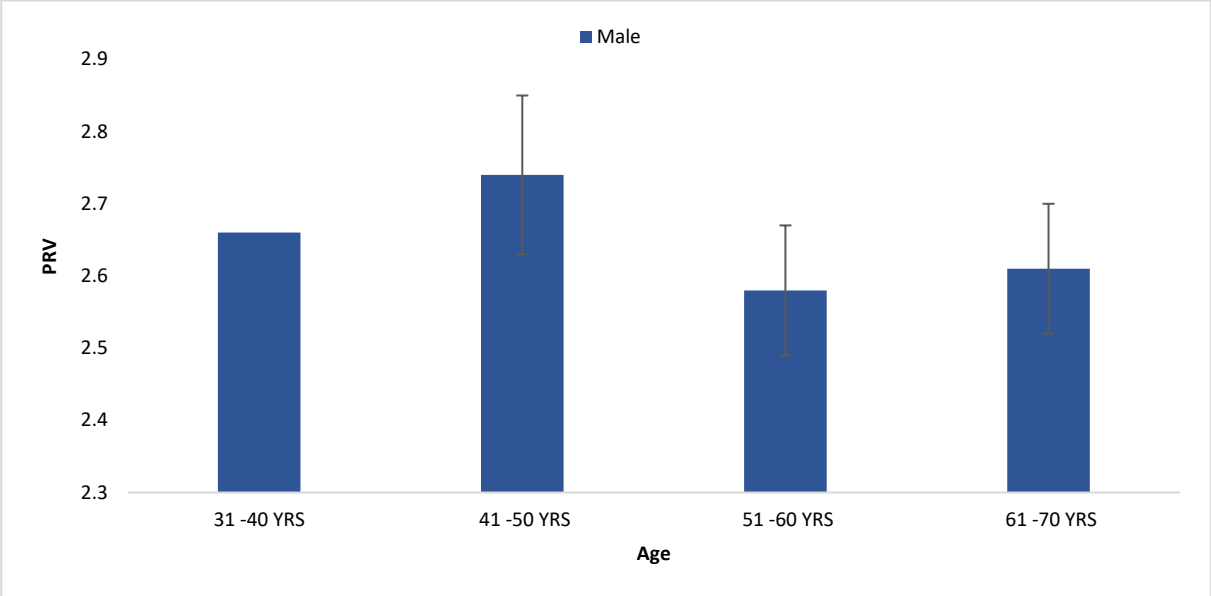

Figure 5: Age based Comparison of Plasma Relative Volume in Male subjects

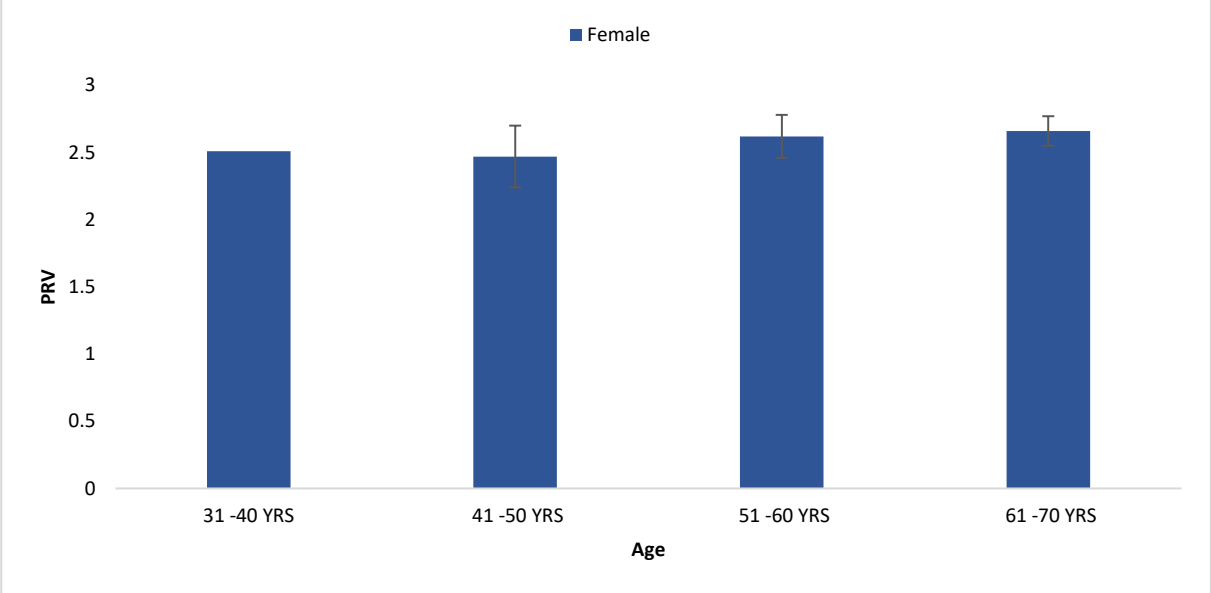

Figure 6: Age based Comparison of Plasma Relative Volume in Female subjects

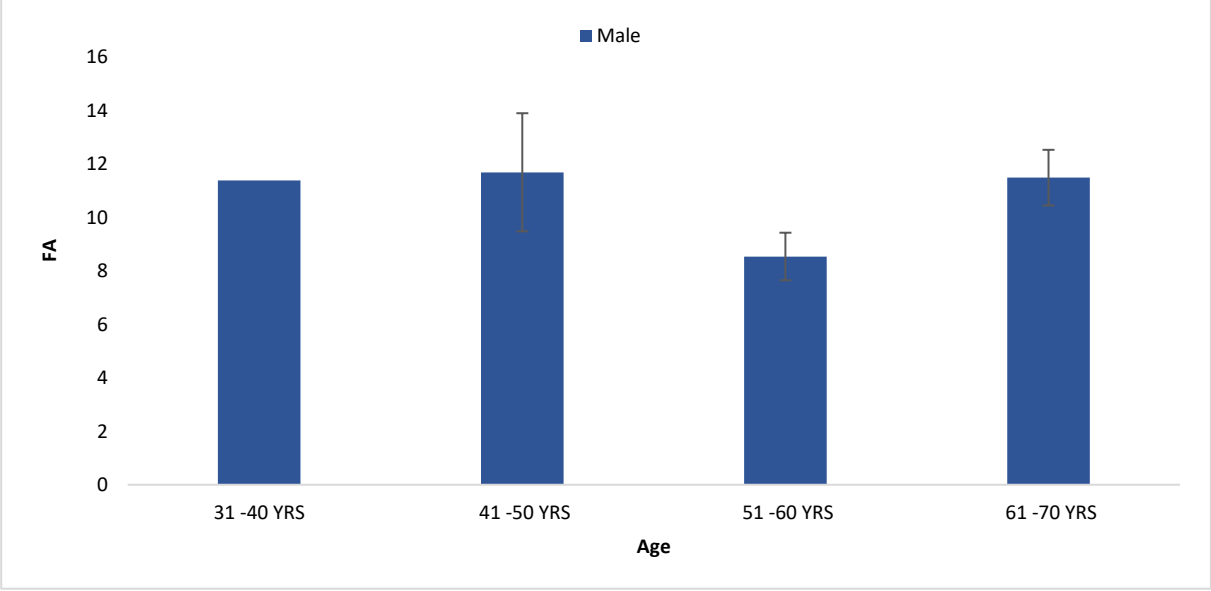

Figure 7: Age based Comparison of Fibrinolytic Activity in Male subjects

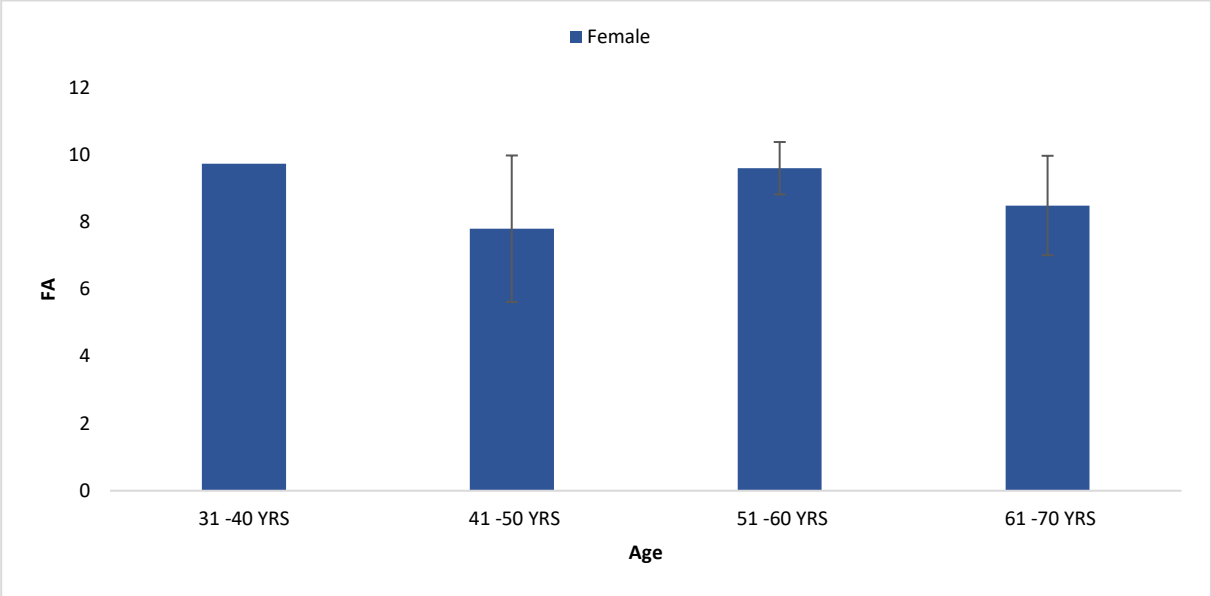

Figure 8: Age based Comparison of Fibrinolytic Activity in Female subjects

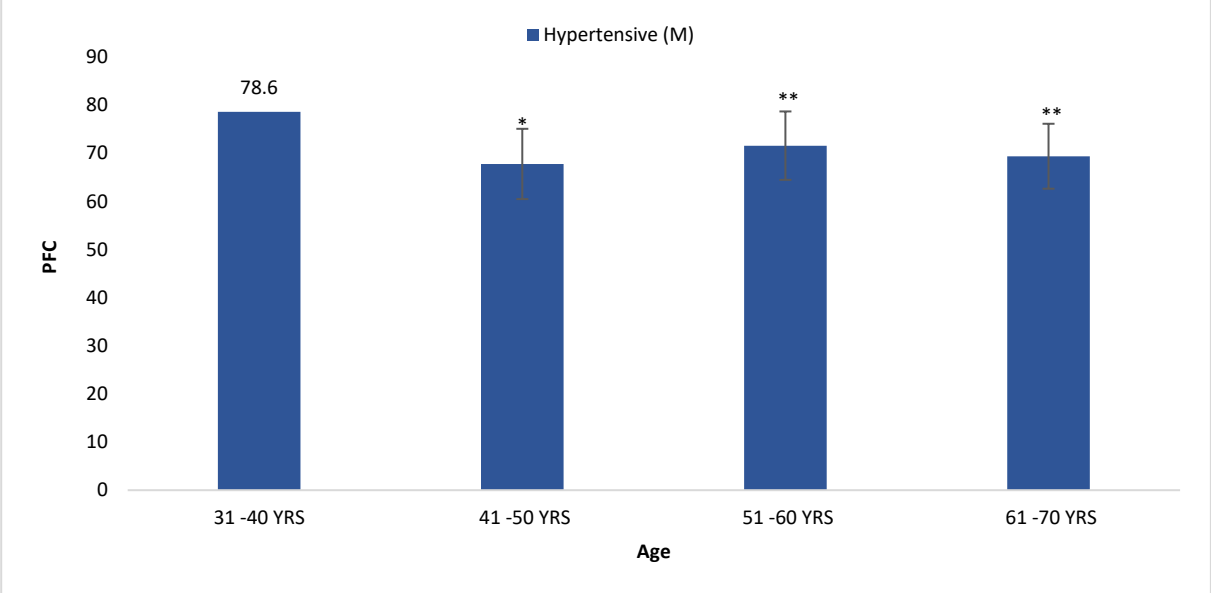

Figure 9: Age based Comparison of Plasma Fibrinogen Concentration in Male subjects

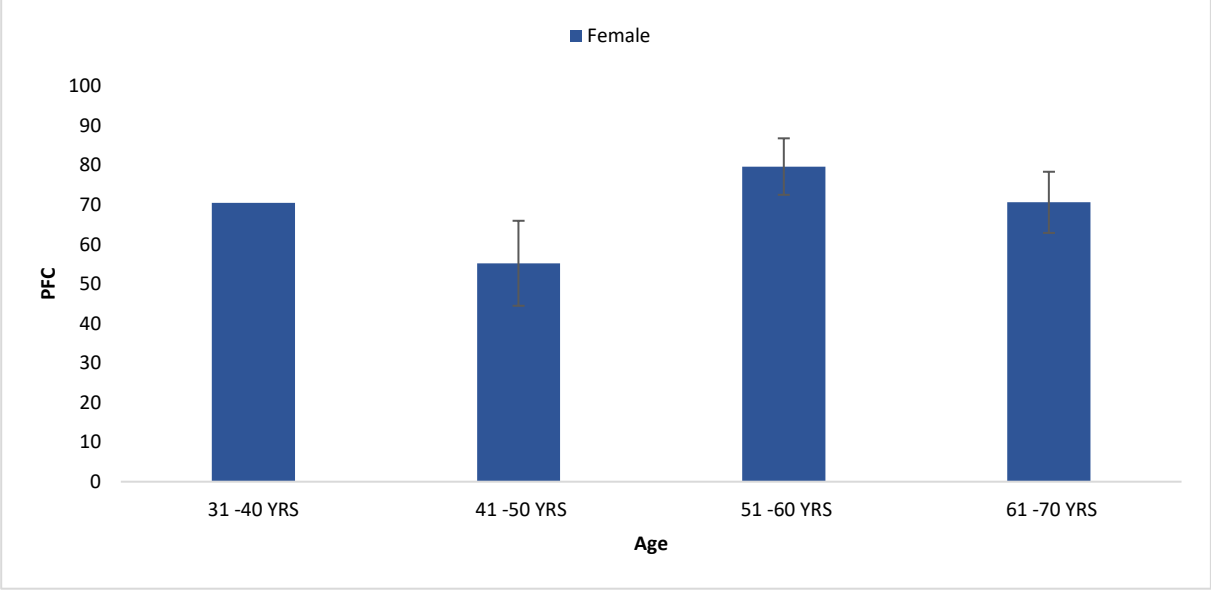

Figure 10: Age based Comparison of Plasma Fibrinogen Concentration in Female subjects

DISCUSSION

In this study, age and gender based comparisons were made of hemorheological characteristics of human subjects. A total of One hundred and forty (140) participants of seventy two (72) males and sixty eight (68) female subjects were ethically approached from the staff and students' community of the University of Benin, Edo state, Nigeria.

From our result, males showed a significantly ($p<0.05$) higher values in most hemorheological variables than their female counterparts across diverse age groups, this finding ascertained most available reports as to gender based increment in hemorheological variables of males than females, suggestive that this could be a contributory factor to the development of elevated blood pressure in males than the females.

Again, observations from figures V and VI shows Relative plasma viscosity (RPV) and plasma fibrinogen concentration (PFC) to have be significantly increased ($p < 0.05$) with age, proving highest in males than the females. However, this value apparently remained the same for females of between 40-51 years as compared with the male folks. Also, packed cell volume (PCV) and Relative whole blood viscosity were also higher among male compared to females but the increase was significant, even though fibrinolytic activity was also observed to be significantly ($p < 0.05$) higher for males compared with their female counterpart. Also, Plasma viscosity noticeably caused an increase in plasma protein density, whilst increasing. However, dissimilar proteins showed variable effects plasma viscosity rates; even though there were huge correlations in plasma globulin, viscosity and fibrinogen levels, with albumin levels returning little or no effect on the plasma viscosity. Relative plasma viscosity is an important haemorheological parameter in Nigerians, and has been shown to Increase plasma viscosity in hypertensives; which in turn result from higher fibrinogen levels due to chronic-phase protein reaction, possibly associated with the increased catecholamine release in hypertension. Plasma viscosity is frequently elevated, due to alterations in the plasma protein content.

Worth stressing again is the observation that Whole blood relative viscosity was significantly higher in younger male compared with the older male folks, similar result was also reported by Ighoroge and Dapper. For females, whole blood relative viscosity was noticeably higher in young, than older females even though this proved to be statistically insignificant. Whole blood relative viscosity was significantly higher in middle aged females than most males of other age brackets; the reason is not clear. Whole blood relative viscosity was highest in subjects in age group 61 to 70 years

For packed cell volume (PCV), study observed a higher value n young male and females than their older counterparts upon comparison; but, this difference was statistically insignificant, contrasting with previous report of Neuhaus *et al*, whose study observed that PCV had great difference that makes viscosity difficult to compare without considering the hematocrit level. Male subjects of both age groups had hematocrite values that were significantly higher than that of females; this is likely because blood viscosity is primarily determined by monthly blood loss in women with obvious effects on haematocrit levels that causes decrease of between 1 to 3 oz of blood. The effect on RBC deformability may be less obvious. Because of monthly bleeding, a woman makes more new blood cells than a man. Her blood contains about 80% more young blood cells and about 85% fewer old blood cells.

Fibrinogen, a plasma protein, contributes more than other proteins to plasma viscosity in healthy subjects this contribution is greatly increased in disease states, particularly in hypertension. In this study Plasma fibrinogen concentration was significantly ($p<0.01$) higher in middle aged male and females than their correspondingly lower and higher aged counterparts. This observation concurs with the previous reports from various studies, but there was no significant different between age groups in both gender. The basis for increased fibrinogen concentration in patients across age and gender lines is not clear. But it is suggested that, plasma volume tends to be lower in older aged humans when compared to the young and middle aged. This haemo-concentration may cause abnormally increased plasma fibrinogen. It may be argued that the increase in plasma fibrinogen level was simply due to a response to stress as fibrinogen is known to be an acute phase reactant.

CONCLUSION

Current study has established a significant difference in the hemorheological variables of male and female adults in both across age groups. From the study, a statistically insignificant difference was seen in all, but whole blood viscosity across gender and age groups, with slight increase in all variables for young and middle aged subjects than the older generation. However, none of the parameters was significantly different across age groups, suggestive that the parameters do not differ across age group in the male participants. This could be a suggestive reason for the increased blood viscosity under high shear circumstances.

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