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# ASSESSMENT OF HEMORHEOLOGICAL ALTERATIONS AS ATHEROSCLEROSIS RISK FACTORS

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## ABSTRACT
The study of hemorheology is of great significance in the area of medical research. The present study was carried out to investigate the association of hemorheological properties including blood viscosity, hematocrit, fibrinogen concentration, RBC aggregation and deformability with atherosclerosis risk factors such as diabetes, obesity and hyperlipidemia. The patients (n=97) presenting with risk factors of atherosclerosis were 49.54±1.42 years, had BMI 30.98±0.65 kg/m$^2$, with lipid measures as total cholesterol (TC) 258.92±4.16 mg/dl, triglyceride (TG) 231.07±6.24 mg/dl, HDL 33.37±1.64, LDL 180.46±4.47 mg/dl, VLDL 47.04±1.66 mg/dl, fasting sugar (FS) 193.33±7.67 mg/dl which, except the age, were significantly ($p<0.0001$) higher than the controls; the viscosity (Visc), hematocrit (Hct), fibrinogen (Fib), yield shear stress (YSS), shear rate (SR), rouleax formation intensity (RFI), and filterability index (FI) respectively were 4.84±0.06 cP, 48±0.37%, 0.47±0.005 g/dl, 0.23±0.007 dynes/cm$^2$, 0.046±0.001 s$^{-1}$, 1.76±0.08, 1.10±0.009%, ($p<0.0001$), where the values were expressed as the mean ± standard deviation. Diabetic, obese and hyperlipidemic men had significantly higher TC, TG, VLDL, Hct, Vis, YSS and FI compared to their female counterparts. The YSS was found to increase rapidly with increase in Hct, SR, RFI and FI in atherosclerosis. There was significant association between diabetes, obesity and hyperlipidemia with hemorheological alterations in atherosclerosis and between FS, BMI, lipid profile with hemorheological parameters in controls. Hyperviscosity was significantly associated with low levels of HDL cholesterol with concomitant hypertriglyceridemia, hypercholesterolemia and hyperfibrogenemia in atherosclerosis. The alterations in the flow behavior property of blood is an important factor in the development of atherosclerosis.

**KEYWORDS:** Viscosity, hematocrit, fibrinogen, RBC aggregation, deformability, lipid profile.

## INTRODUCTION
Atherosclerosis, regarded as prolonged inflammation and fibroproliferation of the intima of arterial vessels, is a significant health problem worldwide, leading to cardiovascular and cerebrovascular diseases.[1] There are several atherogenic risk factors affecting the vasculature including obesity, diabetes mellitus, hypertension, hypertriglyceridemia, hypercholesterolemia and low HDL levels. However, in some cases these processes may not be sufficient to explain why blood flow falls to levels low enough to cause tissue infarction from ischemia. The blood flow rate according to Poiseuille law is a function of vessel size and fluid viscosity. Experimental findings implicate increased whole blood viscosity as another possible explanation for decreased blood flow in some clinical situations.[2] This happens particularly when flow is already reduced to minimal on account of raised viscosity of blood as its flow falls and increased viscosity causes increased resistance to flow.[3] Impaired hemorheological conditions leading to abnormal visco-elastic properties of blood, cause insufficient blood supply in atherosclerosis, that depends on the number and properties of the red blood cells including, packed cell volume, fibrinogen concentration, total serum proteins, aggregation and deformability.[4] Generally an increase in hemorheological variables causes hypoperfusion which results in impaired microcirculation.[5] Several studies confirmed the role of classical risk factors in the genesis of atherosclerotic lesions,[6-7] however there is very little information about visco-elastic parameters (blood viscosity hematocrit, fibrinogen, red blood cell aggregation and deformability) as the risk factors. Description of viscosity values unencumbered by secondary influences makes available data for assessment of other patients with enhanced cardiovascular and cerebrovascular risk based on blood hyperviscosity. The relative contributions of hematocrit (Hct), fibrinogen (Fib) on the viscosity of blood provide insights into the mechanisms by which these biochemical parameters effect rheological factors. Besides, a relatively few number of studies examined the association between the hemorheological parameters and

the conventional risk factors towards the development and progression of vascular diseases. There is no report at all on the above two aspects from this region, where the study was undertaken besides there being a scarcity of information from the adjoining states. Together these alterations lead to substantial deterioration of the flow properties of blood, involving whole blood viscosity and its influence on hematocrit, fibrinogen, fasting plasma lipids, red blood cell aggregation and deformability. Thus the current investigation was carried out to measure blood viscosity from healthy adults in order to estimate the viscosity reference values from healthy population; to measure blood viscosity from hyperlipidemic, obese and diabetic for the determination of any association between them; to determine hematocrit, fibrinogen concentrations, yield shear stress (YSS) and shear rate (SR) of blood for evaluating shear-dependent interactions of plasma lipids with erythrocytes in blood rheology and for evaluating the association between hemorheological alteration with the risk factors for atherosclerosis.

## MATERIALS AND METHODS

### *Case selection*

Both male and female patients presenting with risk factors for atherosclerosis attending the OPD of the hospital between 2014 and 2017 were included as the subjects (n=100; 50 males and 50 females) for, the present study, which were carried out in the Department of Physiology and in the Department of Biochemistry, MGM Medical College and Hospital, Kishanganj, Bihar. Atherosclerosis risk were assessed on the basis of the presence of obesity, diabetes and hyperlipidemia. Nonsmoking and healthy subjects (n=97; 50 males and 47 females) will be considered as controls for the study.

Clearance from the Institutional Ethical Committee were obtained prior to the study. A written consent were obtained from the participants. The clinical history of the participants will be recorded in a questionnaire and subjected to necessary clinical examination. All subjects were questioned concerning recent or ongoing diseases, including hyperlipidemia and medications. Any relevant investigation were done as and when required.

### *Exclusion criteria*

Subjects were excluded from this screening if they have known endocrinologic disorders, renal insufficiency, hepatic diseases, or illnesses characterized by an acute-phase response. The regular and recent use of either prescription or nonprescription medications were also reason for exclusion. Those unable to co-operate because of age or mental status and those unwilling to give informed consent were excluded from the study.

### *Visco-elastic measurements*

Blood samples were taken from the cubital vein; viscoelastic parameters such as viscosity, hematocrit, fibrinogen concentration, yield shear stress, shear rate, aggregabilty and deformability were determined. Measurements were carried out at 37.0 ± 0.5ºC within 2 hours after venepuncture.

Viscosity of blood with EDTA were calculated from the time for sample to flow a predefined distance along the Ostwald's capillary viscometer and compared with that of the water of known viscosity and will be expressed as the ratio of the two, the viscosity of water being 1.0 centipoise (cP) units at room temperature. Hematocrit were determined by Wintrobe's method and read from the height of column of RBCs, in the Wintrobe's tube after centrifugation, expressed as % age of blood. Fibrinogen concentration were determined by rapid titration method using 0.25% methylene blue for unstrained visualization of the clot end point.[8] A titer of ≥ 128 indicates fibrinogen content ≥ 0.2 g %, a titer of 64 indicates fibrinogen concentration of 0.1-0.2 g % and a titer of < 32 indicates fibrinogen < 0.1 g %. The yield shear stress (YSS) of blood is the force necessary to initiate flow in immobile state of blood, that was determined based upon the interaction of fibrinogen (Fib) and hematocrit (Hct) on viscosity using the equation: $YSS = 13.5(10^{-6})(Fib)^2(Hct - 6)^3$, as described by Merrill.[10] Although the non-Newtonian characteristics of blood were assumed, shear rate (SR) was estimated indirectly using the equation: $SR = YSS/Viscosity$, by empirically applying the laws of viscosity.[10-12] The different degrees of red cell aggregation were evaluated in terms of rouleaux formation intensity (RFI) due to the effect of plasma factors on the erythrocytes. The RFI were estimated from observations of its occurrence in different microscopical fields, as follows: normal (N), slightly increased (1+), moderately increased (2+) and strongly increased (3+).[13] Red Cell Deformation (RCD), the ability of erythrocytes to change their shape in order to travel through capillary beds were estimated in terms of filterability index (FI) by measuring erythrocyte filterability using micropore membrane; washed erythrocytes, poor in leucocytes and platelets, resuspended in phosphate-buffered saline (PBS), were used for study of RCD.[14]

### *Biochemical measurements*

The biochemical correlates such as fasting blood glucose, lipid profile were estimated using standard methods.

### *Anthropometric measurements*

The weight and height of the control and subjects were measured.

### *Statistical analysis*

All data were evaluated as mean ± SEM (standard error of mean) by student's t-test and chi-square test. The extent of relationship between visco-elastic characteristic of blood with biochemical and parameters were determined by Pearson's correlation coefficient and the association were expressed using regression analysis.

**RESULTS**

A cohort of 100 (50 men, 50 women) healthy nonsmoking adults and 97 (50 men, 47 women) adults with dyslipidemia, obesity and diabetes were subjected to anthropometric measurements, fasting blood analysis for lipid profile and glucose and estimation of hemorheological parameters. Viscosity measurements were made on blood samples at room temperature, using Ostwald viscometer and a 3 ml syringe with its plunger removed and the blood was allowed to flow freely.

Table 1 shows the distribution of blood viscosity values and biochemical variables in healthy adults and in hyperlipidemic, obese and diabetic adults. There was an insignificant difference between healthy males and females regarding age, TC, TG, HDL, LDL, VLDL and fasting sugar (FS) while the BMI and viscosity values were significantly different between the two genders. However, in the hyperlipidemic, obese and diabetic group of adults, there was an insignificant difference in age, HDL, LDL and FS while BMI, viscosity, TC, TG and VLDL were significantly different between males and females. The hemorheological parameters in normal and hyperlipidemic adults is indicated in Table 2. The total values along with the corresponding values in controls and hyperlipidemic men and women has been compared here. The hemorheological parameters including hematocrit, fibrinogen, red blood cell aggregation, deformation showed significant difference between the control and hyperlipidemic groups of males and females except the SR and RFI parameters ($p>0.05$).

The regression analysis of lipid parameters and hemorheological parameters with viscosity in males and females is depicted in Table 3. A significantly high correlation of viscosity with FI in normal and hyperlipidemic males and females, with RFI in hyperlipidemic males and females was obtained ($r>0.9$, $p<0.001$). A moderate level of association was seen between viscosity levels and YSS, RFI, SR in normal males, with YSS and RFI in normal males, while hyperlipidemic males exhibited similar correlation r value ranging from 0.50 to 0.78. The regression model for the analysis of normal and elevated hemorheological parameters with diabetics and non-diabetics, with obese and non-obese individuals is represented in Table 4, which produced significant association between them.

Fig. 1 shows the variation of YSS with Hct and SR in both controls and in atherosclerosis risk groups which indicated a significant correlation of YSS with SR in both groups. The association between YSS and RFI and between YSS and FI in both controls and in atherosclerosis is represented in Fig. 2, which showed a significant but poorer correlation except between YSS and FI in atherosclerosis. The variation of viscosity levels with hemorheological parameters including hematocrit, fibrinogen, red blood cell aggregation, deformation in normal and hyperlipidemic adult males and females is represented in Fig. 3; among all the variables, the RFI values increased steeply with viscosity whereas the slope of the remaining variables were relatively with very lower steeps.

**Table 1: Distribution of blood viscosity values and biochemical variables in healthy adults and hyperlipidemic, obese and diabetic adults**

| Parameters | Healthy adults | | | | Hyperlipidemic, obese, and diabetic adults | | | |
|---|---|---|---|---|---|---|---|---|
| | Total | Men | Women | p value | Total (p value) | Men | Women | p value |
| **Age (years)** | 46.91±1.53 | 47.6±2.19 | 46.22±2.13 | 0.66 | 49.54±1.42 (0.21) | 51.18±2.06 | 47.79±1.92 | 0.24 |
| **BMI (kg/m$^2$)** | 20.99±0.22 | 20.54±0.32 | 21.43±0.29 | 0.04 | 30.98±0.65 (<0.0001) | 29.21±0.6 | 32.74±0.7 | 0.03 |
| **Viscosity (cP)** | 4.06±0.03 | 4.21±0.03 | 3.92±0.04 | <0.0001 | 4.84±0.06 (<0.0001) | 5.26±0.07 | 4.39±0.04 | <0.0001 |
| **TC (mg %)** | 172.74±3.29 | 173.36±3.63 | 172.12±5.49 | 0.85 | 258.92±4.16(<0.0001) | 268.72±5.22 | 248.49±6.02 | 0.02 |
| **TG (mg %)** | 109.62±3.59 | 113.2±4.96 | 106.04±5.14 | 0.32 | 231.07±6.24(<0.0001) | 253.86±10.32 | 206.83±4.58 | 0.0001 |
| **HDL (mg %)** | 46.89±1.48 | 46.7±1.88 | 47.1±2.29 | 0.90 | 33.37±1.64 (<0.0001) | 35.94±3.02 | 30.63±0.88 | 0.10 |
| **LDL (mg %)** | 103.91±3.23 | 104±0.32 | 103.81±4.83 | 0.98 | 180.46±4.47(<0.0001) | 184.19±6.21 | 176.49±6.39 | 0.40 |
| **VLDL(mg%)** | 21.92±0.72 | 22.64±0.99 | 21.20±1.03 | 0.32 | 47.04±1.66 (<0.0001) | 52.37±2.92 | 41.37±0.92 | 0.0007 |
| **FS (mg %)** | 91.75±1.31 | 90.5±1.96 | 93±1.71 | 0.34 | 193.33±7.67(<0.0001) | 193.84±0.14 | 192.79±9.6 | 0.95 |

BMI, Body Mass Index. TC, Total cholesterol. TG, Triglyceride. HDL, High density lipoprotein. LDL, Low density lipoprotein. VLDL, Very low density lipoprotein. FS, Fasting Sugar

**Table 2: Hemorheological parameters including hematocrit, fibrinogen, red blood cell aggregation, deformation in normal and hyperlipidemic adults**

| Parameters | Control | Hyperlipidemic | p value | Control | | p value | Hyperlipidemic | | p value |
|---|---|---|---|---|---|---|---|---|---|
| | Total | Total | | Men | Women | | Men | Women | |
| **Hct (%)** | 42.59±0.24 | 48±0.37 | <0.0001 | 43.8±0.26 | 41.38±0.33 | <0.0001 | 50.68±0.39 | 45.15±0.27 | <0.0001 |
| **Viscosity (cP)** | 4.06±0.03 | 4.84±0.06 | <0.0001 | 4.21±0.03 | 3.92±0.04 | <0.0001 | 5.26±0.07 | 4.39±0.04 | <0.0001 |
| **Fib (g %)** | 0.31±0.005 | 0.47±0.005 | <0.0001 | 0.32±0.006 | 0.34±0.007 | <0.0001 | 0.46±0.006 | 0.48±0.008 | 0.005 |
| **YSS (dyne/cm$^2$)** | 0.07±0.002 | 0.23±0.007 | <0.0001 | 0.062±0.003 | 0.073±0.003 | 0.03 | 0.26±0.01 | 0.19±0.007 | <0.0001 |
| **SR (s$^{-1}$)** | 0.02±0.0006 | 0.046±0.001 | <0.0001 | 0.015±0.0007 | 0.018±0.0008 | 0.0006 | 0.05±0.002 | 0.04±0.0014 | 0.14 |
| **RFI** | 0.26±0.054 | 1.76±0.08 | <0.0001 | 0.14±0.05 | 0.38±0.093 | 0.03 | 1.76±0.115 | 1.74±0.11 | 0.97 |
| **FI (%)** | 0.96±0.006 | 1.10±0.009 | <0.0001 | 0.99±0.006 | 0.93±0.008 | <0.0001 | 1.17±0.01 | 1.08±0.006 | <0.0001 |

Hct, Hematocrit. Fib, Fibrinogen. YSS, Yield shear stress. SR, Shear rate. RFI, Rouleaux formation intensity. FI, Filterability index.

**Table 3: Regression analysis of lipid parameters and hemorheological parameters with viscosity in males and females**

| Subjects | Regression equation | | | |
|---|---|---|---|---|
| | Normal | r | Hyperlipidemic | r |
| **Males** | TC = -16.33 Visc + 242.1 | 0.15 | TC = -1.2076 Visc + 275.08 | 0.02 |
| | TG = 23.004 Visc + 16.369 | 0.15 | TG = -8.4567 Visc + 298.36 | 0.06 |
| | LDL = -14.621 Visc + 165.55 | 0.11 | LDL = -6.7219 Visc + 219.56 | 0.07 |
| | HDL = -6.1704 Visc + 72.676 | 0.11 | HDL = 7.3292 Visc - 2.6271 | 0.17 |
| | VLDL = 4.6007 Visc + 3.2737 | 0.15 | VLDL = -1.503 Visc + 60.275 | 0.05 |
| **Females** | TC = 11.102 Visc + 140.39 | 0.13 | TC = -22.12 Visc + 345.62 | 0.13 |
| | TG = 2.4174 Visc + 99.131 | 0.03 | TG = 0.8244 Visc + 203.21 | $R^2$ = 5E-05 |
| | LDL = 9.2392 Visc + 77.405 | 0.13 | LDL = -21.167 Visc + 269.44 | 0.12 |
| | HDL = 1.354 Visc + 43.2 | 0.04 | HDL = -1.1178 Visc + 35.542 | 0.05 |
| | VLDL = 0.4835 Visc + 19.826 | 0.03 | VLDL = 0.1649x + 40.642 | $R^2$ = 5E-05 |
| **Males** | Fib = 0.0598 Visc + 0.0337 | 0.31 | Fib = 0.0164 Visc + 0.3708 | 0.19 |
| | YSS = 0.0597 Visc - 0.1891 | 0.60 | RFI = 1.5999 Visc - 6.6594 | 0.95 |
| | RFI = 0.9557 Visc - 3.8828 | 0.64 | FI = 0.1418 Visc + 0.4209 | 0.99 |
| | SR = 0.011Visc - 0.0317 | 0.50 | YSS = 0.1101 Visc - 0.3239 | 0.75 |
| | FI = 0.1944 Visc + 0.1766 | 0.99 | SR = 0.0089 Visc + 0.0004 | 0.36 |
| **Females** | RFI = 1.9162 Visc - 7.1287 | 0.78 | Fib = -0.0408 Vis + 0.6646 | 0.20 |
| | FI = 0.2124 Visc + 0.1023 | 0.99 | FI = 0.1789 Visc + 0.2432 | 0.99 |
| | Fib = -0.0058 Visc + 0.3663 | 0.03 | RFI = 3.0262 Vis - 11.522 | 0.97 |
| | YSS = 0.0471 Visc - 0.1117 | 0.54 | YSS = 0.0747 Visc - 0.1343 | 0.42 |
| | SR = 0.0077 Visc - 0.0117 | 0.38 | SR = 0.0076 Visc + 0.0108 | 0.21 |

TC, Total cholesterol. TG, Triglyceride. HDL, High density lipoprotein. LDL, Low density lipoprotein. VLDL, Very low density lipoprotein. Visc, Viscosity. Fib, Fibrinogen. YSS, Yield shear stress. SR, Shear rate. RFI, Rouleaux formation intensity. FI, Filterability index.

**Table 4: Regression analysis of hemorheological parameters with diabetes, obesity, and hyperlipidemia**

| Subject | Hemorheological parameter | Regression equation | | p value |
|---|---|---|---|---|
| | | Non-diabetic with normal hemorheological parameter | Diabetic with elevated hemorheological parameter | |
| **Men** | **Hct** | Hct = -0.0066 FS + 44.402 | Hct = 0.0001 FS + 50.652 | <0.0001 |
| | **Viscosity** | Visc = -0.0009 FS + 4.2952 | Visc = 0.0001 FS + 5.2402 | <0.0001 |
| | **FI** | FI = -0.0002 FS + 1.01 | FI = 4E-06 FS + 1.1663 | <0.0001 |
| | **Fib** | Fib = 0.0006 FS + 0.2326 | Fib = 2E-05 FS + 0.4531 | <0.0001 |
| | **YSS** | YSS = 0.0002 FS + 0.0411 | YSS = 5E-05 FS + 0.246 | <0.0001 |
| | **SR** | SR = 7E-05 FS + 0.0085 | SR = 8E-06 FS+ 0.0458 | <0.0001 |
| | **RFI** | RFI= -0.006 FS + 0.6806 | RFI= -0.0003 FS + 1.8252 | <0.0001 |
| **Women** | **Hct** | Hct = 0.0465 FS + 37.051 | Hct = -0.0021FS + 45.553 | <0.0001 |
| | **Viscosity** | Visc = 0.0052 FS+ 3.4312 | Visc = -0.0003 FS + 4.45 | <0.0001 |
| | **FI** | FI = 0.0012 FS + 0.8263 | FI = = -9E-05 FS + 1.0481 | <0.0001 |
| | **Fib** | Fib = 0.0012 FS + 0.2298 | Fib = -1E-05 FS + 0.4878 | <0.0001 |
| | **YSS** | YSS = 0.0008 FS + 0.0001 | YSS = -4E-05 FS + 0.2009 | <0.0001 |
| | **SR** | SR = 0.0002 FS + 0.0015 | SR = -4E-06 FS + 0.0448 | <0.0001 |
| | **RFI** | RFI= 0.0085 FS - 0.4094 | RFI= -0.0006 FS + 1.8723 | <0.0001 |
| **Men** | **Hemorheological parameter** | **Non-obese with normal hemorheological parameter** | **Obese with elevated hemorheological parameter** | **p value** |
| | **Hct** | Hct = 0.0155 BMI + 43.48 | Hct = 0.0701 BMI + 48.63 | <0.0001 |
| | **Viscosity** | Visc = 0.0014 BMI + 4.18 | Visc = = 0.0126 BMI + 4.90 | <0.0001 |
| | **FI** | FI = 0.0004 BMI + 0.9871 | FI = 0.0016 BMI + 1.1204 | <0.0001 |
| | **Fib** | Fib = 0.0026 BMI + 0.232 | Fib = -0.001 BMI + 0.48 | <0.0001 |
| | **YSS** | YSS = 0.0011 BMI + 0.0395 | YSS = -0.0003 BMI + 0.27 | <0.0001 |
| | **SR** | SR = 0.0003 BMI + 0.0089 | SR = -0.0003 BMI + 0.055 | <0.0001 |
| | **RFI** | RFI= -0.0158 BMI + 0.4637 | RFI= 0.0283 BMI + 0.9327 | <0.0001 |
| **Women** | **Hct** | Hct = 0.0155 BMI + 43.48 | Hct = 0.0701 BMI + 48.63 | <0.0001 |
| | **Viscosity** | Visc = 0.0014 BMI + 4.18 | Visc = = 0.0126 BMI + 4.90 | <0.0001 |
| | **FI** | FI = 0.0004 BMI + 0.9871 | FI = 0.0016 BMI + 1.1204 | <0.0001 |

| | | | | |
|---|---|---|---|---|
| | **Fib** | Fib = 0.0026 BMI + 0.232 | Fib = -0.001 BMI + 0.48 | <0.0001 |
| | **YSS** | YSS = 0.0011 BMI + 0.0395 | YSS = -0.0003 BMI + 0.27 | <0.0001 |
| | **SR** | SR = 0.0003 SBMI + 0.0089 | SR = -0.0003 BMI + 0.055 | <0.0001 |
| | **RFI** | RFI= -0.0158 BMI + 0.4637 | RFI= 0.0283 BMI + 0.9327 | <0.0001 |

Hct, Hematocrit. Visc, Viscosity. Fib, Fibrinogen. YSS, Yield shear stress. SR, Shear rate. RFI, Rouleaux formation intensity. FI, Filterability index.

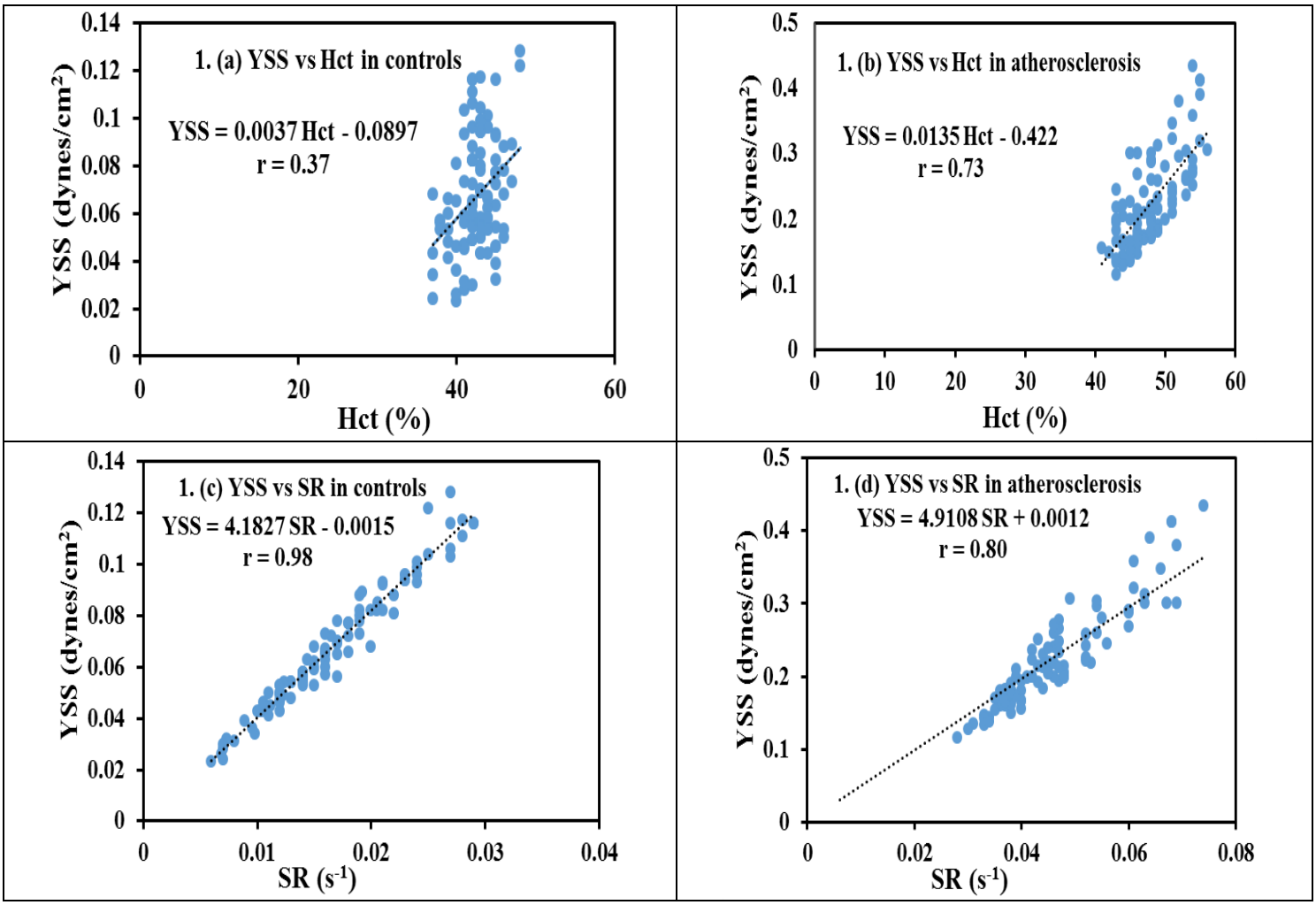
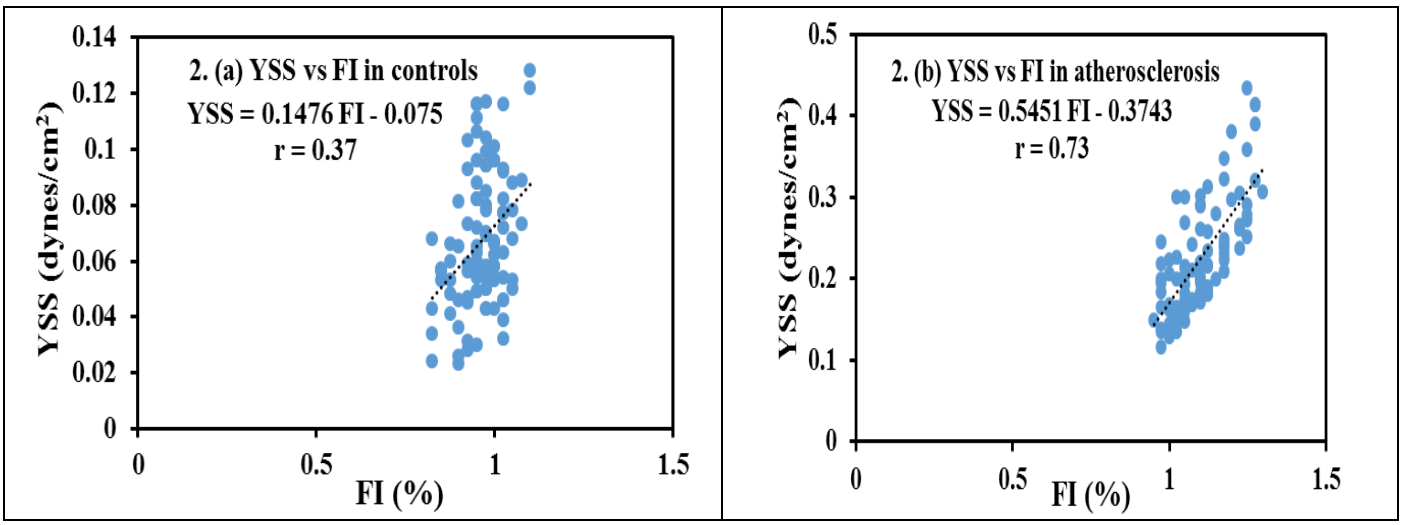



**Figure 1: Variation of yield shear stress (YSS) with hematocrit (Hct) and shear rate (SR) in control and in patients with atherosclerosis risk**



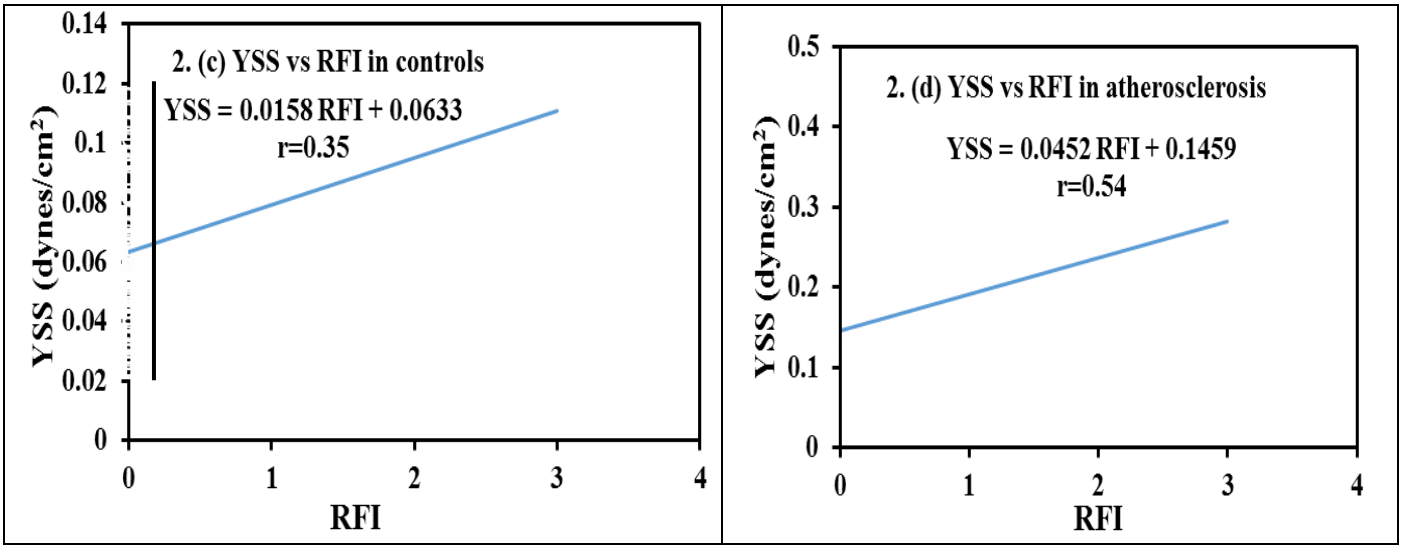



**Figure 2: Variation of yield shear stress (YSS) with Filterability index (FI) and Rouleaux formation intensity (RFI) in control and in patients with atherosclerosis risk**

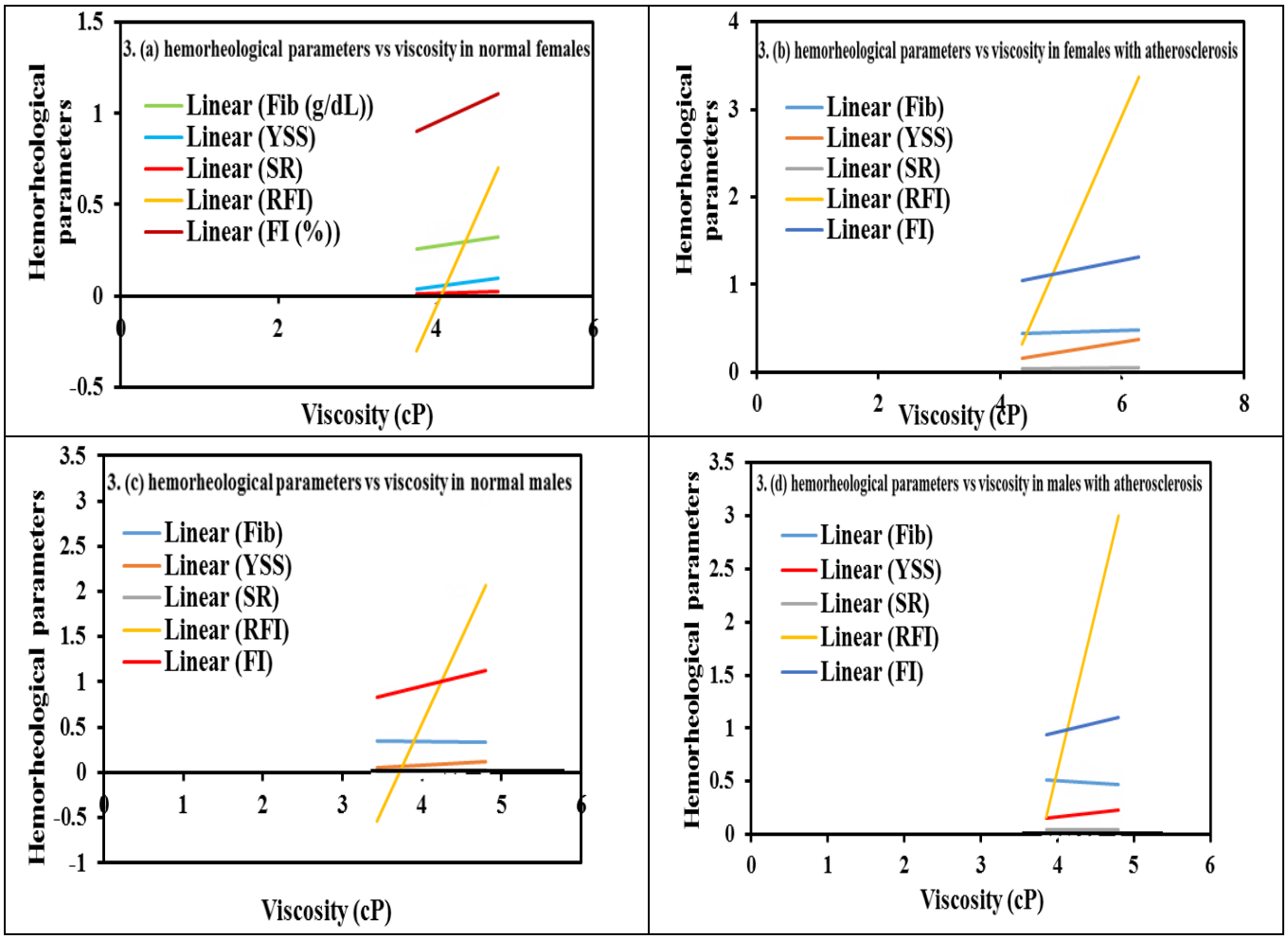



**Figure 3: Variation of hemorheological parameters with viscosity in normal and in patients with atherosclerosis risk**

## DISCUSSION

Based upon the author's knowledge, this is the first report from this region on the issues of blood viscosity values, measured concomitantly from healthy individuals as well as from a population at risk for atherosclerosis. This is also the first documentation of the comparative investigation on the influences of atherosclerosis risk factors such as dyslipidemia, obesity and diabetes on hemorheological variables including hematocrit, viscosity, fibrinogen, red blood cell aggregation and

deformation in order to gain comprehensions into the mechanisms by which the biochemical parameters modulate hemorheology as risk factors in atherosclerosis.

The estimation of blood viscosity in the current study was carried out at room temperature using capillary viscometer and simple syringe method based on time to discharge principle in which the viscosity of blood is given by blood flow time divided by water flow time, considering the viscosity of water being 1.0 cP unit at room temperature.[15,16] This follows from the Hagen-Poiseuille equation which expresses the volume rate of flow V of a fluid as $V = \frac{\pi \Delta P r^4}{8\eta l}$, where, ΔP is the pressure difference between the two ends of the tube, l is the length of the tube, r is the radius of the tube and η is the viscosity of the fluid. Normally, blood viscosity rises < 2% for each $^{o}$C decline in temperature.[17]

This study implicates reference levels for the viscosity of blood that may help in assessing hemorheological profiles (Table 1). The mean blood viscosity for the healthy cohort were relatively lower compared to the hyperlipidemic, obese and diabetic group of adults with values 4.06±0.03 cP and 4.84±0.06 cP ($p<0.0001$) respectively at shear rates of 0.02±0.0006 $s^{-1}$ and 0.046±0.001 $s^{-1}$ ($p<0.0001$) respectively (Table 1). Both normal and abnormal men had significantly higher ($p<0.0001$) blood viscosity values than women being 4.21±0.03 cP in normal men and 3.92±0.04 cP in normal women while 5.26±0.07cP in abnormal men and 4.39±0.04 cP in abnormal women at shear rates 0.015±0.0007 $s^{-1}$, 0.018±0.0008 $s^{-1}$, 0.05±0.002 $s^{-1}$, 0.04±0.0014 $s^{-1}$ respectively (Table 1 and Table 2). There was an insignificant difference between the shear rates in the hyperlipidemic men and women.

The current investigation reports significantly higher ($p< 0.05$) levels of serum total and VLDL cholesterol and triglycerides in men with atherosclerosis risk compared to similar group of women (Table 1). Occurrence of ischaemic cardiovascular disease mortality is greater in men compared to women showing associated blood lipid profile with enhanced atherogenesis.[18] For the entire group, there was a statistically significant although with poor positive correlation between lipid profile variables including TC, TG, LDL, VLDL and whole blood viscosity while elevations in the viscosity of blood was inversely associated (significant with poor negative correlation) with HDL cholesterol (Table 3), however, this observation has been attributed to concurrent hypercholesterolemia and hypertriglyceridemia.[19] The development of atherosclerosis may be induced by raised blood viscosity levels through enhanced platelet adhesion to the subendothelium, increased protein permeation into the arterial wall and through modification of local shear forces, at atherogenesis locations.[20]

Significantly higher levels of fibrinogen ($p<0.006$) were observed in women as compared to men (Table 2) and the fibrinogen concentrations were significantly associated with blood viscosity although with low but positive correlation coefficient, at the corresponding shear rates (Table 3). These findings are consistent with those reported in literature. Fibrinogen due to its anisometric property, high density and almost neutral charge endows maximum viscosity to the blood.[21] Several reports have advocated the role of fibrinogen as a significant risk factor for the early development of atherosclerosis through several mechanism, comprising increased fibrin production, decreased fibrin breakdown, elevated platelet aggregation, and blood viscosity.[20-22]

The hematocrit concentration is the main factor influencing blood viscosity. In the present study, the blood viscosity values were associated significantly ($p < 0.0001$) with very high positive correlation with Hct levels in both control and abnormal cohort of individuals at risk of atherosclerosis (Table 3). Viscosity of blood varies with changing Hct. When the Hct increases, the friction between the consecutive layers of the blood rises. With increasing Hct, the viscosity of blood escalates significantly. The viscosity of blood is about 3 cP at 40% hematocrit and 10cP at 65% Hct; within a range of 60 to 70% Hct, the viscosity of blood reaches 10 times that of water; however, the hematocrit shows logarithmic relationship with blood viscosity within the hematocrit concentrations range of 35% to 55%; at cell concentrations exceeding 10%, hematocrit is directly proportional to the viscosity of blood.[23,24] Over the range of 0-50% Hct, the viscosity of blood is related to the hematocrit H by an equation $\eta = \eta_p\{1 + 0.025H + 7.35 \times 10^{-4}H^2\}$, where $\eta_p$= plasma viscosity.[25]

When the hematocrits of two samples are widely different, their viscosities are compared by measuring viscosity index that provides a correction for the effect of hematocrit due to the fact that the relationship of blood viscosity with Hct is linear between 30% and 50% Hct.[19] The blood viscosity is related to plasma viscosity by the following equation: $\eta = \eta_p$ (1+2.5 Hct). Rosenblum (1968) studied the effect of different anticoagulant on the viscosity of blood and reported that blood viscosity was elevated by anticoagulants which permitted erythrocyte shrinkage manifested by decreased hematocrit of blood.[26]

Blood exhibits a yield shear stress (YSS) defined as the force required to start movement in a stationary column of blood in order to overcome the prevailing van der Waals-London forces and Coulombic forces among the different strata of blood.[27] Within a temperature range of $10^{0}$C to $37^{0}$C, the YSS value of human blood is 0.05 dyne/cm$^2$.[28] The YSS was significantly higher ($p <0.0001$) in the subjects with atherosclerosis risk compared to the healthy individuals with mean ± SEM

values 0.23±0.007 dyne/cm$^2$ and 0.07±0.002 dyne/cm$^2$ respectively; the YSS were also significantly greater ($p < 0.0001$) in males than the females with atherosclerosis risk (Table 2). The YSS was significantly associated with blood viscosity in both normal cohort and in subjects with atherosclerosis risk with high correlation coefficient in both males and females except in females with atherosclerosis risk, there was a significant but low correlation between YSS and viscosity (Table 3). The YSS was found to increase rapidly with increase in Hct ($r=0.73$, $p<0.0001$), SR ($r=0.80$, $p<0.0001$), FI in atherosclerosis (Figure 1 and 2). The higher the yield stress of the static blood, the higher the driving pressure needed to get the flow started again.

Blood viscosity is particularly dependent on shear rate, being high at low shear rates and low at high shear rates,[29] which poses challenges to the precise measurement of vascular wall shear stress, referred to as the frictional force per unit area acting tangentially to the arterial wall. Measurements of blood viscosity at very low shear rates is important as it provides information on blood critical threshold of stress, or blood yield shear stress, an indicative of the development of weak percolating physical gel, leading to extremely hindered flow states and even to compaction states,[30] based upon the cohesive forces between them, which is important for the assessment of the presence of any microvascular ischemic diseases.[31] Within the Hct range of 30-50%, the YSS is given by $\tau_y = A(H - H_c)^3$, where the critical hematocrit $H_c$ below which the YSS is insignificantly small is between 4 and 8% and the fibrinogen concentration dependent constant A has a value of approximately 0.6-1.2 $X$ $10^{-6}$ for normal blood.[26] The YSS for blood Hct 40% is normally 0.04 dynes/cm$^2$. The YSS showed significantly high ($r>0.7$, $p<0.0001$) positive correlation with Hct, SR, FI in atherosclerosis, while it was significantly associated although by low correlation coefficient with Hct, FI and RFI in healthy subjects; YSS was related significantly by very high ($r=0.98$, $p<0.0001$) positive correlation with SR in controls whereas there was moderate association between YSS and RFI in patients with atherosclerosis risk ($r=0.54$, $p<0.0001$). The YSS was found to increase rapidly with increase in Hct, SR, RFI and FI in atherosclerosis (Figure 1 and Figure 2).

RBCs can adhere together forming stacks of coin like structures called rouleaux. RBC aggregation, a phenomenon usually present in normal physiology, is an important hemorheological factor. The rouleax formation intensity (RFI) were 1.76±0.08 in patients presenting with risk factors of atherosclerosis which were significantly ($p<0.0001$) higher than the controls (Table 2). RFI in patients with risk of atherosclerosis were significantly associated with viscosity in both males and females ($r>0.94$, $p<0.001$) while in normal subjects RFI was also significantly correlated with viscosity but with a lower coefficient ($r= 0.64$ in males, $r=0.78$ in females, $p<0.001$) (Table 3). The RFI increased rapidly with increase in viscosity in both healthy persons and in patients presenting with risk factors of atherosclerosis (Figure 3). The blood flow through post capillary venules are subjected to low shear rates, where aggregation of RBCs (depending on RBC membrane surface and suspending medium properties) occur leading to increased blood viscosity, though the forces producing aggregation are weak that under high shear rates tend to break up leading to lesser blood viscosity, an effect known as shear thinning of blood. The red cell aggregation dissimilarities between normal and subjects with atherosclerosis risk can be described chiefly in relation to the effects of altered fibrinogen concentration leading to changed plasma proteins metabolism due to diabetes, obesity stimulating vascular diseases in such patients.

The RBCs also tend to undergo change in their shapes when subjected to shear forces, a property known as red cell deformability (RCD), which is an important factor effecting blood flow, especially in microcirculation. There are several factors effecting RCD including hypoinsulinemia, diabetes, hyperosmolarity, RBC membrane lipids, its glycation, viscosity, increased sorbitol levels.[32] RCD confers discoidal biconcave form to the RBCs when freely suspended in medium suggestive of high surface area: volume ratio. The filterability index (FI) were 1.10±0.009% in patients presenting with risk factors of atherosclerosis which were significantly ($p<0.0001$) higher than the controls (Table 2). Diabetic, obese and hyperlipidemic men had significantly ($p<0.0001$) higher FI compared to their female counterparts (Table 2). FI in both normal subjects and in patients with risk of atherosclerosis were significantly associated with viscosity with very high correlation coefficient in both males and females ($r=0.99$, $p<0.001$) (Table 3). RCD effects blood viscosity and microvascular resistance at moderate to high shear rates.[24] Filtration methods are generally used to evaluate RCD properties as filtration of RBCs through artificial narrow pores is similar to *in vivo* microcirculation.[33] The whole blood filterability is an index of erythrocyte deformation that in the present study was determined on washed RBC resuspended in phosphate-buffered saline and was obtained by weighing the amount of RBC filtered through micropore membranes, the filtration of RBC implemented under the influence of gravity only, and the quantity of the RBC filterate is compared with the PBS filterate through the same membrane.

There was a significant association ($<0.0001$) of hemorheological parameters with fasting sugar levels in both males and females (Table 4) assessed in terms of comparative regression model development between non-diabetics with normal hemorheological parameters and diabetics with elevated hemorheological parameters. Patients with diabetes but with normal renal function had early impairment in RCD and progressive impairment in

RCD was, irrespective of the presence or absence of diabetes, associated with kidney dysfunction.[32]

There was significant association of normal and elevated hemorheological parameters with obese and non-obese individuals (Table 4). A study on the hemorheological parameters in isolated obesity showed a significant increase in RFI or erythrocyte aggregation in obese population when compared to the controls while the FI or the red blood cell deformability was significantly decreased, with concomitant increases in Vis and Fib level.[34] It was proposed in a study that partly secondary risk factors such as physical fitness, prolonged psychoemotional stress, obesity etc, were involved in the genesis of atherosclerotic lesions and influence rheological properties of blood quantified by blood viscosity, red cell filterability and red cell aggregation, in inter- and intraindividual comparisons of such parameters with fitness having a significant influence on blood fluidity, stress and excessive obesity leading to deterioration in blood fluidity and red cell filterability.[35] A significantly high correlation of viscosity with FI in normal and hyperlipidemic males and females, with RFI in hyperlipidemic males and females was obtained ($r>0.9$, $p<0.001$) (Table 3).

In the present study, there was significant association between diabetes, obesity and hyperlipidemia with rheological alterations in blood ($p<0.0001$) and between FS, BMI, lipid profile with hemorheological parameters in controls ($n=100$) ($p<0.0001$ in all controls except in female controls with HDL vs Hct, $p=0.018$; YSS vs RFI, $p=0.002$; SR vs RFI, $p=0.0003$) and an insignificant association between YSS vs RFI in healthy males ($p=0.123$), Fib vs RFI in healthy females ($p=0.7$), increased VLDL vs increased Hct ($p=0.57$). In diabetic females, there was insignificant variations between fasting sugar levels and hemorheological changes by F-test ($p=0.97$). Blood viscosity was significantly higher in dyslipidemia patients with LDL >140 mg/dl, TG >150 mg/dl or HDL < 40 mg/dl compared with patients without dyslipidemia, although Hct and FI did not differ between the two groups; LDL and TG were positively correlated with blood viscosity, but HDL was negatively correlated.[36] The patients with hyperlipidaemia had blood viscosity higher than healthy people but without any difference in the FI between the groups.[37] There was no difference in the FI among the smokers and in patients with hyperlipidaemia while blood viscosity calculated at 4 shear rate between 0.37 and 69.5 $sec^{-1}$ increased in subjects compared to the controls.[1]

The clinical importance of hemorheological evaluation in cardiovascular risk (diabetes, obesity, dyslipidemia) involves reference levels achieved from a healthy population. Results from the present study provide evidence to suggest that changes in hemorheological profile may be important risk factors for the development of early atherosclerosis leading to the development of coronary artery disease. Thus evaluation of rheological parameters of blood may help documentation and categorization of at risk patients for atherosclerotic vascular disease and its complications.

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