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Fibrinogen, Relative Blood Viscosity and Haematocrit in the three trimesters of Pregnant Albino Wistar Rats

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ABSTRACT: This study evaluated the changes in relative blood viscosity during pregnancy in Albino wistar rats. Twenty (20) female wistar rats were divided into two groups, control group (n=5) and pregnant group (n=15). The pregnant group was further subdivided into 1st trimester group, 2nd trimester group and 3rd trimester group respectively, with five rats (n=5) in each group.. Female rats in their oestrous cycle were mated with fertile male rats over night; a closed vagina with the presence of sperms in the vagina smear was taken as day 1 of pregnancy. Blood was collected from the control group and the trimester group for the assessment of fibrinogen, relative blood viscosity and haematocrit. Results showed that Fibrinogen was significantly higher in the 3rd trimester compared to the 1st and 2nd trimesters and the control. Relative blood viscosity was significantly higher in the 1st trimester compared to the 2nd, 3rd trimesters and control. There was no significant difference ($p>0.05$) in haematocrit value in the control and pregnant groups. Haematocrit showed a highly significant correlation ($r=0.98$; $p<0.05$) in the 3rd trimester of pregnancy. Conclusively, blood viscosity is highest in the 1st trimester, and starts to decrease as pregnancy advances.

Keywords: Haematocrit, Fibrinogen, viscosity, pregnancy, trimesters

Introduction

Pregnancy is associated with many physiological changes; including cardiovascular system, metabolic, respiratory, renal and haematologic systems (Costantine, 2014). Haematologic changes includes: increase in blood cells, increase in clotting factors and fibrinogen (Pacheco *et al*., 2013). Blood viscosity is said to be the inherent resistance of blood to flow, and a direct measure of the ability of blood to flow through the vessels (Holsworth and Wright, 2012) and is affected by factors such as haematocrit, red blood cell deformability, red blood cell aggregation, fibrinogen, plasma viscosity (Kesmarky *et al*., 2008). Increased blood viscosity is a major biological parameter that has been linked with all other major cardiovascular risk factors such as metabolic syndrome, high blood pressure, elevated low density lipoprotein (Sloop, 1996). Highly viscous blood pounding against the walls of the blood vessels leads to abrasion of the single-cell layer of the intima in the carotid, pulmonary and coronary arteries (Kensey and Cho, 2007). The body responds with a protective adaptation, creating plague which eventually calcifies in an effort to protect the blood vessel and in the long run, this causes increased turbulence and an ever narrowing channel for blood flow thereby increasing the vascular resistance (Kensey and Cho, 2007).

Complications of pregnancy (such as preeclampsia, intrauterine growth retardation) have been seen to be associated with elevated blood viscosity (Holsworth and Wright, 2012). The decreased oxygen carrying capacity of higher viscous blood affects cognitive function, as well as the functions of tissues to which robust oxygen delivery is essential (such as the placenta) (Kameneva *et al*.,1999). The works of Eguchi *et al*. (1994) and Olusegun *et al*.

(2011) reported no significant change in relative blood viscosity during pregnancy. According to Sakai *et al* (1992), whole blood viscosity levels were found to be significantly lower from the 20 to 31 weeks of gestation than those in the other periods of gestation.

Significant reduction in relative blood viscosity during pregnancy was observed by Imoru and Emeribe (2008) while an increase in blood viscosity during pregnancy were reported by Ezechukwu *et al*. (2014). The conflicting results in the various reports maybe due to differences in methodology and experimental design. With these conflicting reports over time, this study was carried out to investigate the changes in Relative blood viscosity in each trimesters of pregnancy and also to evaluate the relationship between haematocrit, fibrinogen and relative blood viscosity.

Materials and Methods

***Ethics*:** Procedures used in this work is in accordance with ethical principles for medical research involving experimental animals. Cervical dislocation was done without the use of anesthetics (such as chloroform), to get an accurate measurement of Fibrinogen (an active phase reactant).

***Study design*:** young rats (30-60 g) were acquired from anatomy animal house in the University of Benin. They were housed in clean cages and fed daily to attain experimental weight (from 150 g). Gestation in rats is about 20-22 days with an average of 21 days. 1st trimester is the first 7 days, 2nd trimester is from day 8 to day 14 and third trimester is from day 15 to day 21.

Materials: Female rats, metabolic cages, rat chow, EDTA bottles, plain sterile bottles, Reagent (sodium citrate, calcium chloride), stand and clamp, 5 ml syringe and a 1 ml syringe with a 21G needle.

***Method*:** Twenty (20) female virgin albino wistar rats were used. They were divided into two (2) major groups; non pregnant control group (n=5), pregnant group (n=15). The pregnant group was further subdivided into 3 groups; first trimester group (n=5, 1-7 days), second trimester group (n=5, 8-14 days) and third trimester group (n=5, 15-21 days). At the last day of each trimester of pregnancy, blood was collected from the rats with a 5 ml syringe by cervical dislocation. 2.5 ml was collected into a plain bottle containing 0.25 ml of 3.8 % sodium citrate which was used to access Fibrinogen concentration and 2.5 ml into EDTA bottle to access relative blood viscosity (RBV) and haematocrit. Plasma Fibrinogen concentration was assayed for using the Ingram clot weight method (Ingram, 1961). Haematocrit was measured using routine lab techniques; a microhaematocrit centrifuge and a microhaemaatocrit reader. RBV was measured using the syringe method (Reid and Ugwu, 1987).

***Statistics*:** Results are expressed as means±S.E.M. The mean values of each subgroup in the pregnant group and the control were compared by one way ANOVA and LSD. Correlation and regression were done to find the association between fibrinogen, haemotocrit and RBV in each trimester of pregnancy using SPSS statistical package. A p-value at ($P<0.05$) was considered significant.

Results

The fibrinogen concentration of the third trimester (2.4±0.25 g/L) was significantly higher ($p<0.05$) than that of the first (1.26±0.17 g/L) and second (1.56±0.36 g/L) trimesters and the control group (1.2±0.2 g/L) (Table 1).

Table 1: Haematocrit, fibrinogen concentration and relative blood viscosity in the three trimesters of pregnancy of albino Wistar rats.

	Control	First trimester	Second trimester	Third trimester
Hematocrit (%)	35.05±**1.38**	35.48±**1.0**	35.29±**2.80**	34.49±**1.38**
Fibrinogen Concentration (g/L)	1.2±**0.2**	1.26±**0.17**	1.56±**0.36**	2.4± **0.25***
Relative Blood Viscosity	5.47±**0.80**	8.85±**0.12****	6.09±**0.73**	5.56±**0.63**

Means ±S.E.M: standard error of the mean; n=5 (* Significant at ***P 0.02)*** (** significant at ***P 0.005***)

The relative blood viscosity was significantly higher ($p<0.01$) in the first trimester of pregnancy compared to the other two trimesters and the control group. This shows that there was a reduction in relative blood viscosity as pregnancy advances. There was no significant difference ($p>0.05$) in the haematocrit values of both pregnant and control group (Table 1).

There was a significant correlation ($p<0.05$) between blood viscosity and haematocrit in the third trimester of pregnancy (Figure 3). There was no significant correlation ($p>0.05$) between blood viscosity and haematocrit in the first (Figure 1) and second (Figure 2) trimesters of pregnancy. There was also no significant correlation ($p>0.05$) between blood viscosity and fibrinogen concentrations in each trimesters of pregnancy (Figures 4-6).

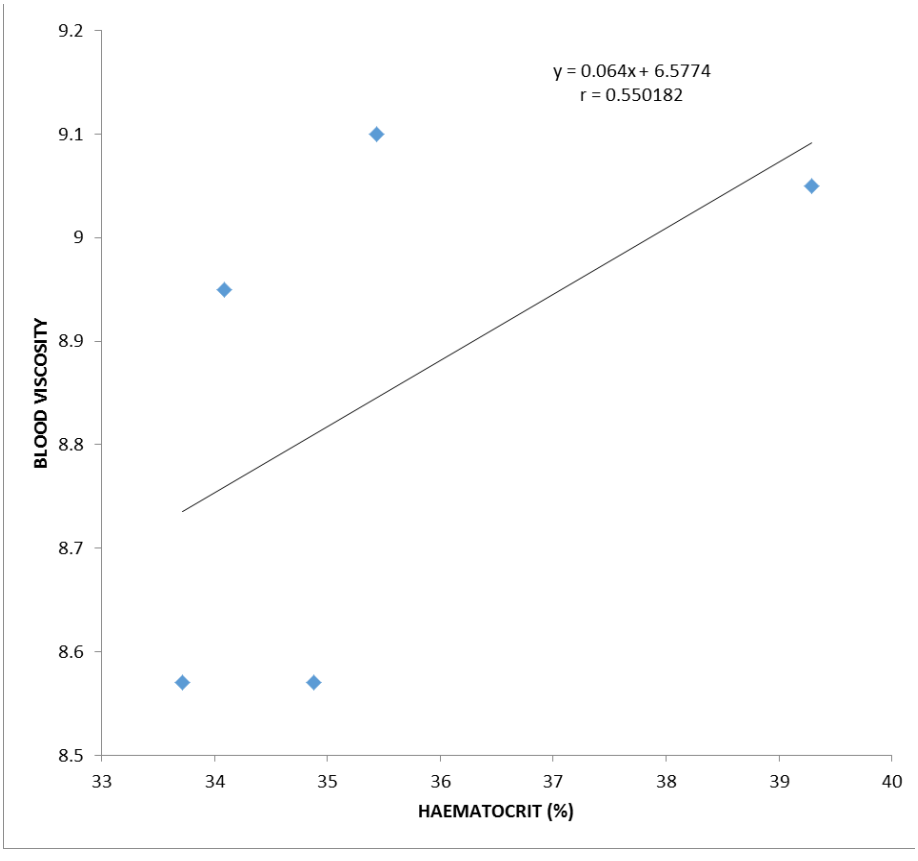

Figure 1: Relationship between relative blood viscosity and haematocrit in the first trimester. There was no significant correlation between the relative blood viscosity and haematocrit (*P 0.34*)

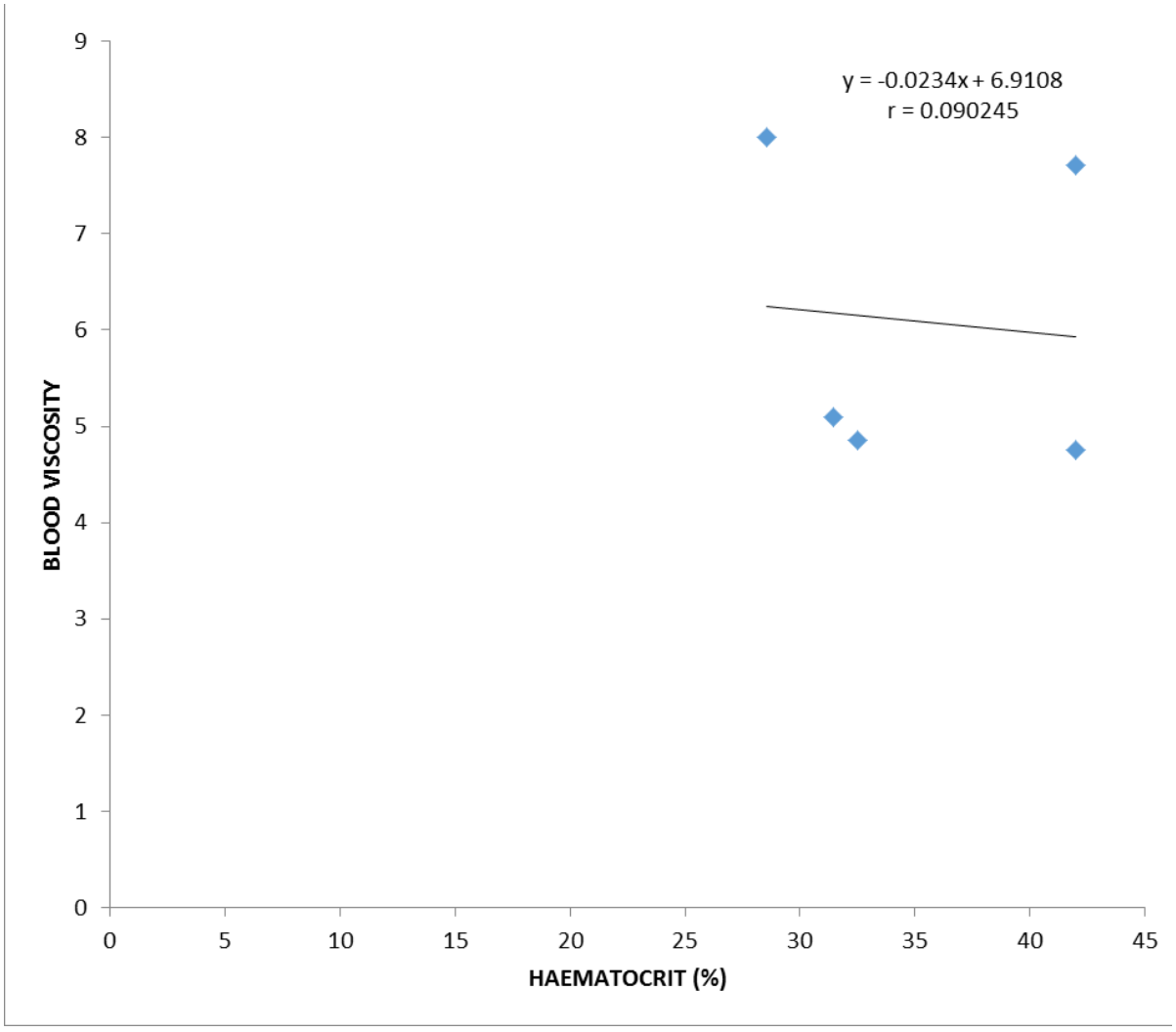

Figure 2: Relationship between blood viscosity and haematocrit in the second trimester. There was no significant correlation between the relative blood viscosity and haematocrit (*p 0.88*)

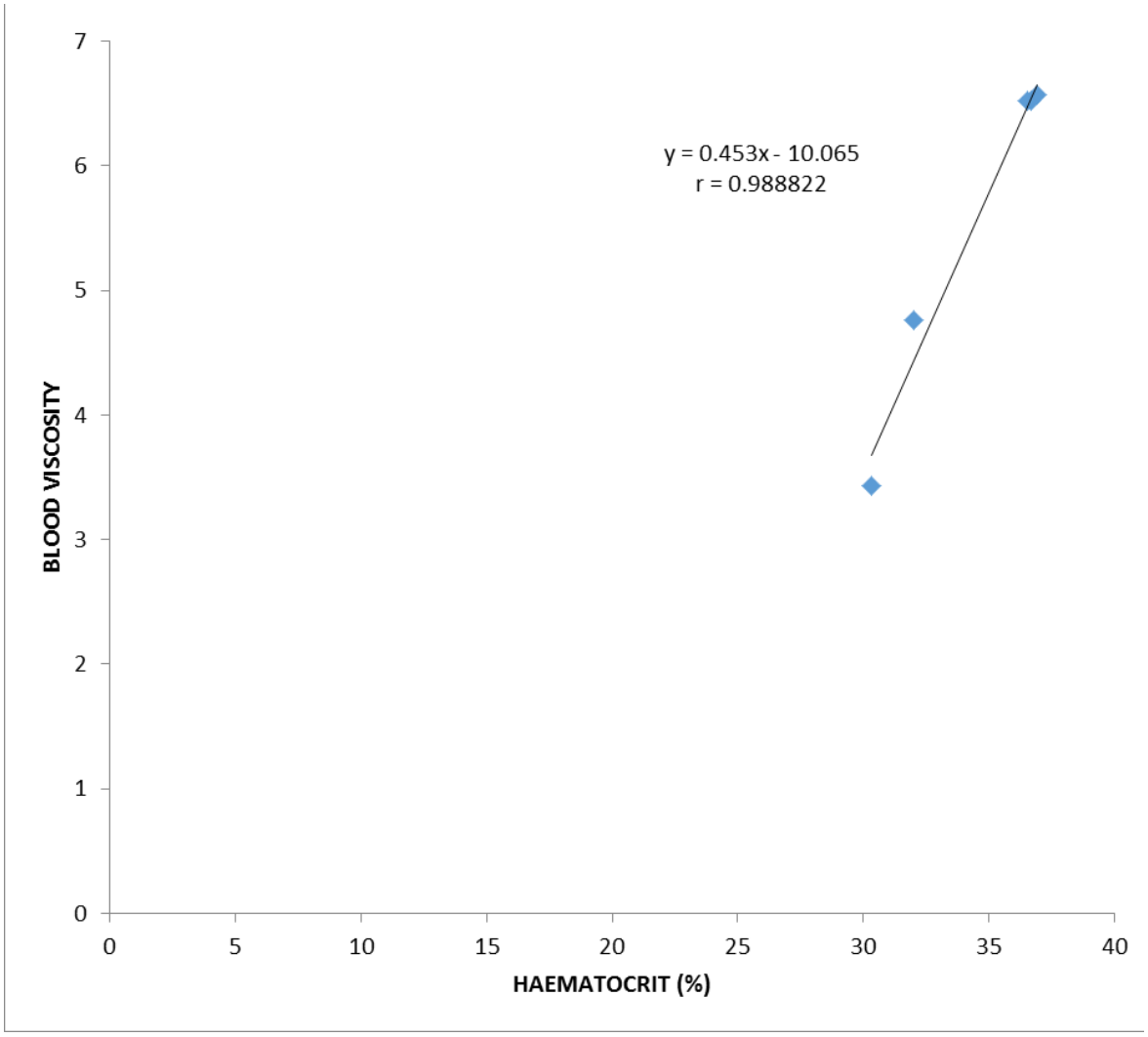

Figure 3: Relationship between blood viscosity and haematocrit in the third trimester. There was a significant correlation between the relative blood viscosity and haematocrit (*P 0.001)* -highly significant). The value of blood viscosity was dependent the haematocrit concentration.

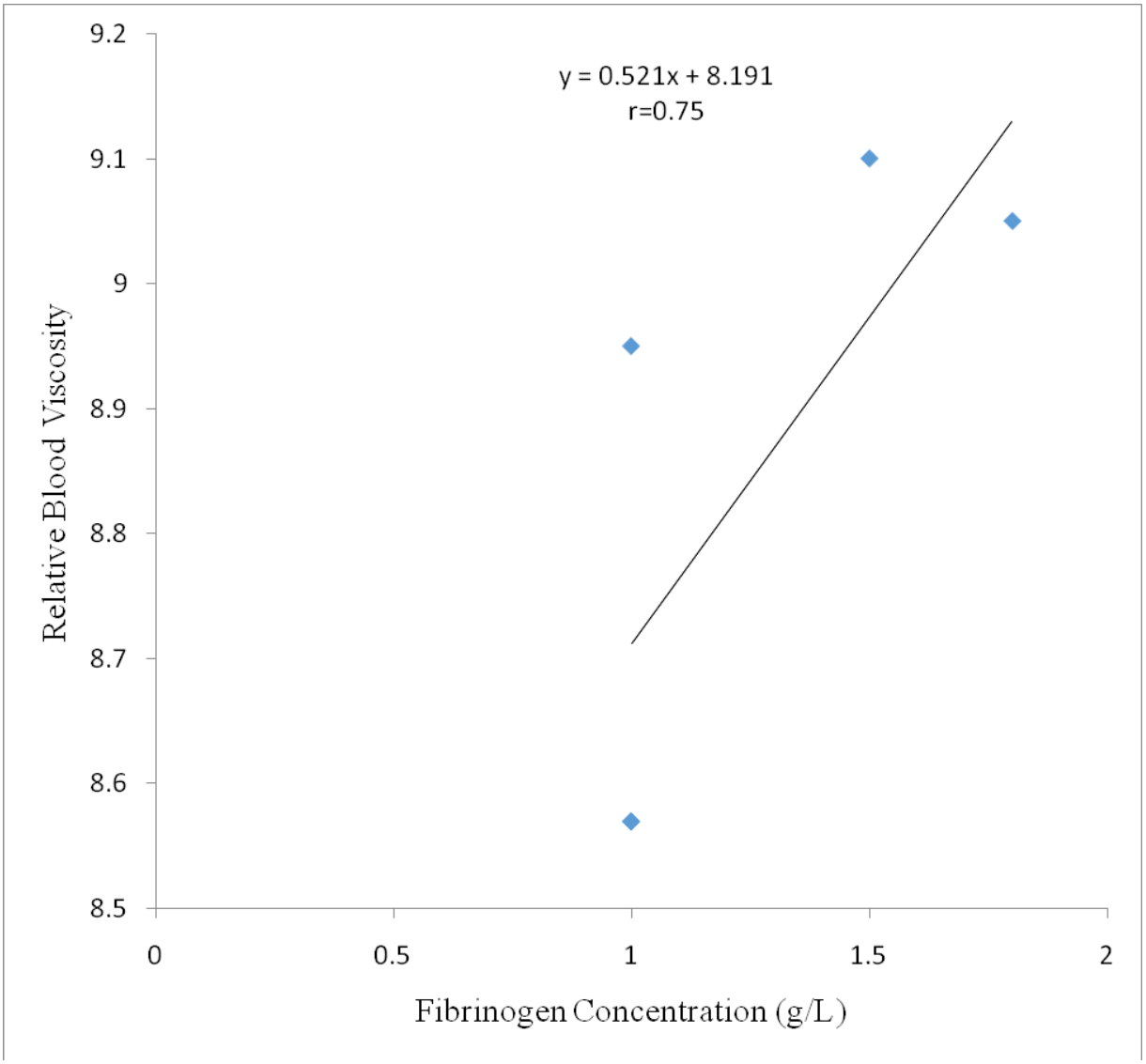

Figure 4: Relationship between blood viscosity and fibrinogen in the first trimester. There was no significant correlation between the relative blood viscosity and fibrinogen concentration. (*P 0.15*)

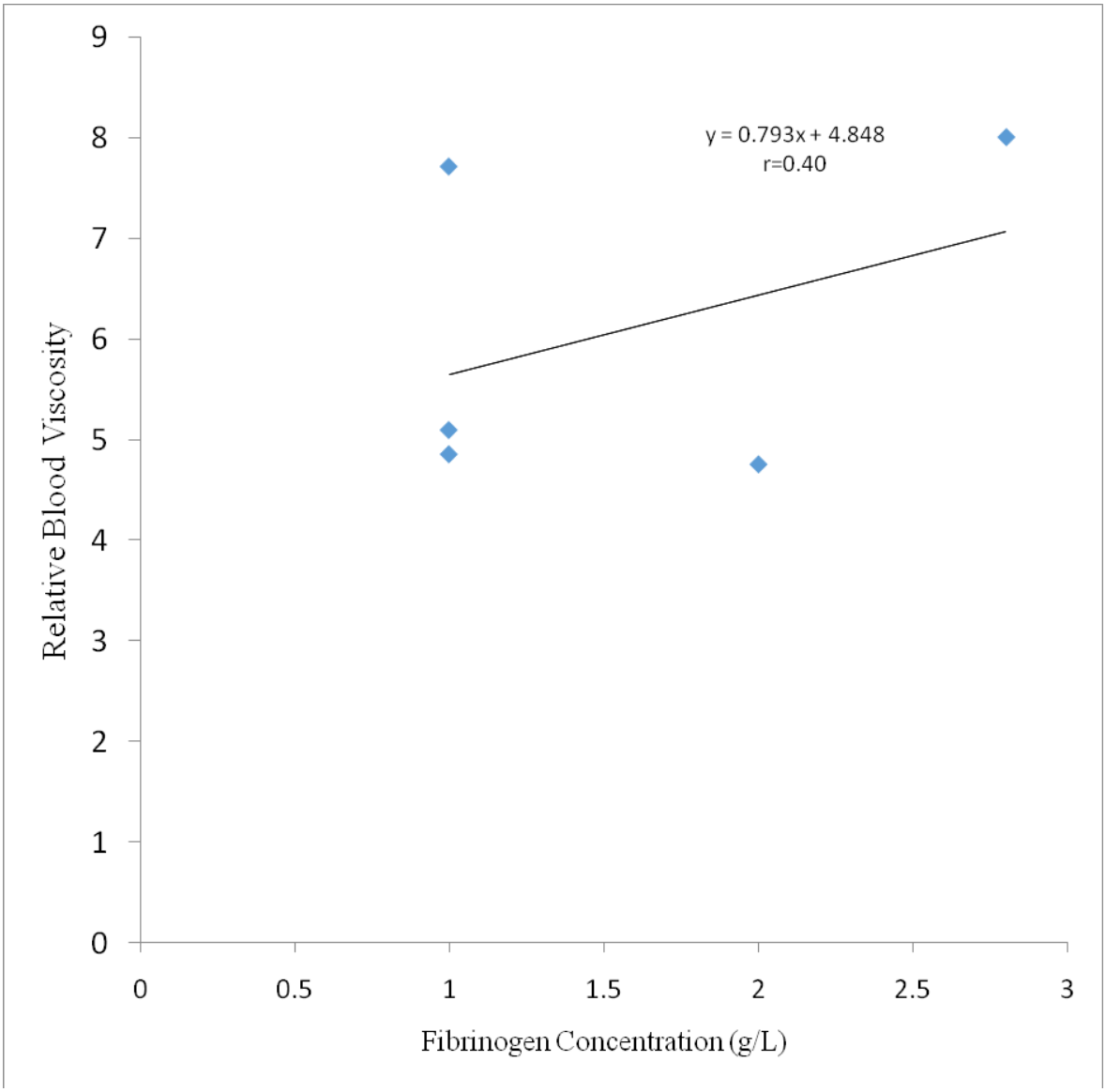

Figure 5: Relationship between blood viscosity and fibrinogen in the second trimester. There was no significant correlation between the relative blood viscosity and fibrinogen concentration (*P 0.50*)

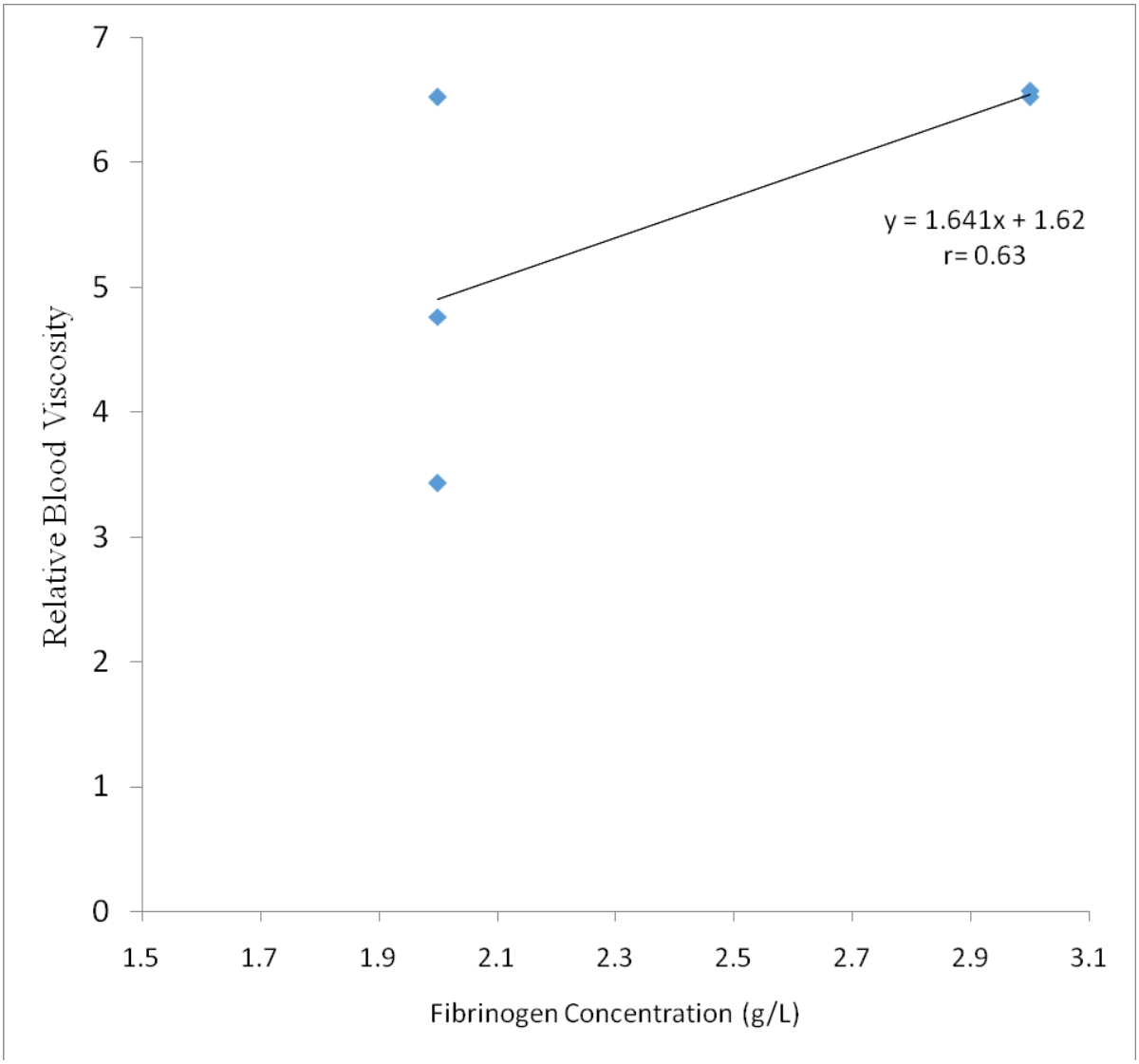

Figure 6: Relationship between blood viscosity and fibrinogen in the third trimester. There was no significant correlation between the relative blood viscosity and fibrinogen concentration (*P 0.25*)

Discussion

The results obtained showed that fibrinogen concentration increased significantly (*P 0.02,* Table 1) as pregnancy advances with the third trimester having the highest value (2.4±0.25 g/L). This agrees with other works that has been done in pregnancy (Pacheco *et al*., 2013). The increased fibrinogen concentration could be due to the increase in its synthesis which has been associated with its utilization in the uteroplacental circulation (Imoru and Emeribe, 2009). Low levels of fibrinogen can be indicative of a systemic activation of the clotting system, with consumption of clotting factors faster than synthesis, a condition known as disseminated intravascular coagulation (Pacheco *et al*., 2013). The relative blood viscosity (RBV) of the first trimester (8.85± 0.12) was significantly (*P>0.005*) higher than the second trimester (6.09 ± 0.73), third trimester (5.56 ± 0.63) and the control group (5.47 ±0.80). This is in line with the work of (Kametas *et al*., 2001), that blood viscosity increase by 10 % during the first trimester of pregnancy in humans. This increase maybe due to an increase in red blood cells, coupled with a lesser increase in plasma

volume in the first trimester (Niraj and Chandraharan, 2012). Imoru and Emeribe (2008) also observed a reduction in relative whole blood viscosity in pregnancy and that this could also be due to marked increase in plasma volume.

Studies have shown that plasma volume increase by 10-15 % in the first trimester and as much as 45-50 % (Alaa *et al*., 2013) in the third trimester and this may be accountable for the decrease in relative blood viscosity seen in the second and third trimesters of pregnancy. The third trimester of pregnancy had the least Haematocrit value compared with the other trimesters of pregnancy and the control group, but the value was not statistically significant (*P 0.98;* Table 1).

Usually, pregnancy is known to be associated with decrease in haematocrit concentration as pregnancy advances. This has been attributed to the greater increase in plasma volume (about 45-50 %) than the increase in red blood cells (15-20 %) thereby resulting in haemodilution (Pacheco *et al*., 2013) and this haemodilution may provide survival advantage to women during pregnancy and childbirth, since the less viscous blood improves uterine and intervillous perfusion, while the increased red cell mass, coupled with increased uterine blood flow, optimizes oxygen transport to the fetus (Pacheco *et al*., 2013). A highly significant correlation was seen between haematocrit and RBV during the third trimester (r=0.98, *P 0.001*). This shows that haematocrit contributes strongly to the RBV during the third trimester.

High maternal haematocrit and hemoglobin values have been associated with adverse pregnancy outcome such as preeclampsia, intrauterine growth retardation (IGR), while low maternal haematocrit has been seen to significantly increase the risk of low birth weight and preterm delivery (Nasiri-Amiri *et al*., 2007). Some studies also showed that women who do not exhibit a fall in hemoglobin during pregnancy have a higher incidence of complications such as preeclampsia and still birth (Stephansson *et al*., 2000).

The fibrinogen concentration increase as pregnancy advances. Despite this increase in the concentration of fibrinogen, there was no significant correlation between fibrinogen and RBV in the three trimesters of pregnancy. Some studies have shown greater increase in fibrinogen and a significant correlation in pathological conditions such as preeclampsia, diabetes mellitus (Alaa *et al*., 2013). The increase seen may not be contributing much to RBV, but as one of those physiology changes that take place in pregnancy and this increase has been seen to contribute to the hyper coagulation state which may provide a survival advantage by minimizing blood loss after delivery, but it also predisposes the pregnant mother to higher risks for thromboembolism (Pacheco *et al*., 2013).

Conclusively, blood viscosity during pregnancy is not constant, it is highest in the first trimester of pregnancy, and starts to decrease as pregnancy advances. Hence, blood viscosity when continually elevated in pregnant women could serve as an early warning for pregnancy complications. Also, haematocrit evaluation during pregnancy is important as it can be used as a marker for changes associated with blood viscosity.

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