African Journal of Cellular Pathology 2:1:14-18 (2014)
The Official Journal of the Society for Cellular Pathology Scientists of Nigeria
www.ajcpath.com

HEMORHEOLOGICAL ALTERATIONS IN NORMAL PREGNANCY

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ABSTRACT

Aim: To determine the hemorhelogical alterations in normal pregnant women in Benin City, Nigeria.

Methods: Subjects aged between 18 and 42 years participated in the study. Patient consent form was obtained from 240 pregnant women on antenatal visit and 80 aged matched healthy individual on routine checkup. The pregnant women were divided into three groups according to trimesters. Those with complications were excluded from the study. C reactive proteins and fibrinogen concentrations were estimated using enzyme-linked immunosorbent assay method. Erythrocyte sedimentation rate was estimated using the Westergren method. Relative plasma viscosity was estimated using the simple syringe and needle apparatus.

Results: There was a significant increase ($P< 0.05$) in FC, RPV, CRP and ESR at stage 1 when compared with stages 2, 3 and 4.

Conclusion: The elevation of fibrinogen concentration, relative plasma viscosity, C reactive protein and erythrocyte sedimentation rate are associated with normal physiological adaptive mechanism of the blastocyst to get itself engraft onto the wall of the endomentrium from where it can obtain nourishment.

Keywords: Adaptive immunity, Fibrinogen, Endomentrium, Acute phase proteins.

INTRODUCTION

Normal pregnancy is postulated to be a state of physiological activation of the innate immune response with increased synthesis of acute phase protein (Romero et al., 2001). The haemostatic system plays an important role in the success of pregnancy and in the process of implantation and placentation. Implantation of the fertilized egg into the uterine decidua establishes a contact between the fetus, the placenta and the maternal circulation. This contact between placenta and maternal circulation is crucial for the success of pregnancy. Pro-thrombotic changes and thrombosis may interfere with these processes leading to miscarriage (Kupfermine et al., 1999). Fibrinogen is a 340-Kd soluble glycoprotein. The plasma content of fibrinogen is synthesized in the

liver (Iwaki et al., 2002). The primary physiological role of fibrinogen is in haemostasis. In the final step of the coagulation cascade, fibrinogen is converted to fibrin, with the formation of a fibrin clot. The first step in this conversion is the thrombin mediated cleavage of fibrinopeptides A and B from the fibrinogen α and β chains; the residual molecule is referred to as fibrin monomer (Blomback et al., 1978). Fibrinogen also have important roles in other pathophysiological processes, apart from clot formation in the haemostatic process. They also play a major role in infection (McRitch et al., 1991), wound healing (Kuijper et al., 1957;Martin et al., 1997) and clot retraction (Gartner and Ogilvie 1988).This study was aimed at determining the hemorhelogical alteration in normal pregnant women in Benin City.

MATERIALS AND METHODS

Subjects aged between 18 and 42 years participated in the study. Patients' consent form was obtained from 240 pregnant women on antenatal visit and 80 aged matched healthy individual on routine checkup. The pregnant women were divided into three groups according to trimester. Those with complication were excluded from the study. Ethical approval was obtained from Lahor medical and research laboratory, Benin City, Edo state.

Blood sample collection

Six ml of venous blood samples were taken from the antecubital vein by venapuncture. 3ml was put into an ethylene diamine tetra acetic acid (EDTA) container while the remaining 3ml was put into a plain container and allowed to clot. It was subsequently centrifuging at 750xg for 15minutes to obtain the serum. The serum was immediately aliquoted into Eppendorf and stored at -20°C.

Quantitative fibrinogen concentration (FC) estimation method

The principle of the double antibody sandwich ELISA method as described by GenWay Biotech, Incorporation, Catalog number 40-288-22856.

Procedure

Fifty micro litres of fibrinogen standard or sample(s) was added per microplate and covered with a sealing tape. It was incubated at room temperature for 2 hours. The solution was discarded and microplates washed four times with 1X wash solution. 50ul of 1X biotinylated anti-human fibrinogen was added to each microplate and incubated at room temperature for one hour with gentle shaking. The microplates were washed as described above. 50ul of 1X Streptavidin conjugate was added to each microplate and incubated for 30 minutes. The microplates were washed as described above. 50ul of Tetramethylbenzidine one step substrate was added to each microplate and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50ul of stop solution was added to each microplate. Tetramethylbenzidine substrate solution was added to the wells and color was developed. The intensity of the color was measured at 450 nm wavelength using stat fax® 4700 micro strip reader

Relative plasma viscosity estimation (RPV)

This compares the flow time of 1ml of plasma to flow time of equal volume of distilled water under gravity in a vertical column at 37°C. Distilled water and samples were brought to 37°C in an incubator, distilled water was carefully drawn into a vertical 1ml syringe and fitted to a retort stand in an incubator, and the plunger was carefully removed to allow the water to flow out. The time it took the water to flow completely out of the syringe was recorded in seconds; this was run in duplicate to obtain a mean value. The samples were treated the same manner. Plasma viscosity expressed as the relative plasma viscosity is the ratio of the flow time of 1ml of plasma (T_P) to the flow time of the same volume of distilled water (T_W) at 37°C as previously described by (Reid and Ugwu, 1987)

C reactive protein estimation (CRP)

Enzyme linked immunosorbent assay was used in determining the level of C reactive protein in the serum. The assay employs an antibody specific for C reactive protein coated on a 96-well plate.

Procedure
One hundred micro litres of C reactive protein standard or samples was added per microplate and covered with a sealing tape. It was incubated at room temperature for 2½ hours. The solution was discarded and microplates washed four times with 1X wash solution. 100ul of 1X Biotinylated anti-human CRP detector was added to each microplate and incubated at room temperature for one hour with gentle shaking. The microplates were washed as described above. 100ul of 1X Streptavidin conjugate was added to each microplate and incubated for 30 minutes. The microplates were washed again as described above. 100ul of Tetramethylbenzidine one step substrate was added to each microplate and incubated for 30 minutes at room temperature in the dark with gentle shaking. 50ul of stop solution was added to each microplate. Tetramethylbenzidine substrate solution was added to the wells and color was developed. The intensity of the color was measured at 450 nm wavelength using stat fax® 4700 micro strip reader as previously described by (Ehiaghe et al., 2013)

Erythrocyte sedimentation rate (ESR) estimation
A well mixed EDTA freshly collected anticoagulated blood was diluted 1:4 with 3.8% trisodium citrate. Diluted samples were drawn to the 200mm mark of the Westergren tube. The tube was set vertically on an ESR rack and a stopwatch started as soon as the tube was set on rack. The results of the test were read after one hour. The height of the clear plasma above the column of the sedimented red cells, to the nearest 1mm was recorded as the ESR in mm/hr. (Lewis, 2006).

Statistical Analysis
All results were presented as mean $\pm$ standard deviation and analyzed using one way analysis of variance (ANOVA) and Turkey – Kramer Multiple comparison test using SPSS – 18.0 statistical program. P values $\leq$ 0.05 were considered significant.

RESULTS
The mean± standard deviation of fibrinogen concentration, relative plasma viscosity, reactive protein and erythrocyte sedimentation rate at stages 1 to 4 are shown in Table 1. There was a significant increase (P< 0.05) in FC, RPV, CRP and ESR at stage 1 when compared with stages 2, 3 and 4.

Table 1: The mean± standard deviation of FC, RPV, CRP and ESR of pregnant women at different trimesters

Parameters	1 N=80	2 N=80	3 N=80	4 N=80
FC(g/l)	2.5±0.02	3.5±0.03	4.2±0.02	5.0±0.01
RPV	1.3± 0.05	1.5±0.02^{A}	1.8±0.01^{B}	2.9±0.01^{C}
CRP(ng/ml)	3.0±0.01	15.0±0.01^{A}	12±0.02^{B}	8.4±0.02^{C}
ESR (mm/hr)	4±0.01	13±0.01^{A}	16±0.01^{B}	20±0.02^{C}

Key:
1=Control group
2=1st trimester
3=2nd trimester
4=3rd trimester
N=Number of sample
A=Significant (P < 0.05) comparison between stage 1 and 2
B=Significant (P < 0.05) comparison between stage 1 and 3
C=Significant (P < 0.05) comparison between stage 1 and 4

DISCUSSION

The significant (P< 0.05) elevation of fibrinogen concentration, relative plasma viscosity reactive protein and erythrocyte sedimentation rate may be attributed to normal physiological adaptive mechanism of the blastocyst to engraft itself onto the wall of the endomentrium where it derives nourishment. This is in line with these findings. Normal pregnancy is often referred to as a hypercoagulable state (Stirling et al., 1984), which is associated with many changes in the haemostatic system; these changes are considered to be in preparation for the maintenance of the placental functions which occurs during pregnancy. Such changes protect women from the haemostatic challenges during delivery and prevent excessive bleeding (Prico et al., 2005). The haemostatic system plays an important role in the success of pregnancy and in the process of implantation and placentation. Implantation of the fertilized egg into the uterine decidua establishes a contact between the fetus, the placenta and the maternal circulation. This contact between placenta and maternal circulation is crucial for the success of pregnancy. Pro-thrombotic changes and thrombosis may interfere with these processes leading to miscarriage (Kupfermine et al., 1999). Pregnancy is a hypervolaemic state characterized by about 40% increase in plasma volume which invariably results in the reduction in plasma viscosity (Kametus et al.,2001).This is a unique and useful hemorheologic adaptation of pregnancy that prevents excessive elevation of blood viscosity despite increased fibrinogen, thereby preventing adverse circulation outcomes in the macro and micro vessels, particularly in the placental bed, which has a sensitive low shear circulation (Kametus et al.,2001). Normal pregnancy is postulated to be a state of physiological activation of the innate immune response with increased synthesis of acute phase protein. These proteins could alter the flow characteristics of blood by altering the protein concentration (Romero et al.,2001). The initial rise in relative plasma viscosity can be attributed to the increased level of fibrinogen, a major determinant of blood viscosity and flow (Kametus et al., 2001;Robins et al., 2003;Tommasso et al.,1991). Complications such as pregnancy induced hypertension and intra uterine growth resistance are associated with abnormal increase in fibrinogen concentration and relative plasma viscosity (Heilman and Siekmann, 1989). Elevation of C reactive protein during normal pregnancy plays a vital role in the maintenance of pregnancy because of its pro and anti inflammatory properties. It may also convey protection to the fetus because of its role in the phagocytosis of micro-organisms (Pitiphat et al., 2005) and activation of the complement cascade (Fogler and Lindsey, 1998).

Conclusion

The elevation of fibrinogen concentration, relative plasma viscosity, C reactive protein and erythrocyte sedimentation rate are associated to normal physiological adaptive mechanism of the blastocyst to engraft itself onto the walls of the endomentrium where it can derive nourishment.

REFERENCES

Blomback B, Hessel B, Hogg D, Therkildsen L (1978). A two-step fibrinogen-fibrin transition in blood coagulation. Nature 275(5680), 501-505

Ehiaghe FA, Agbonlahor DE, Tatfeng YM, Onikepe F, Oviasogie FE, Ehiaghe JI (2013). Serum C reactive protein level in type 2 diabetes mellitus patients attending diabetic clinic in Benin City, Nigeria. Journal of diabetes mellitus. 3 :(4) 168-171.

Fogler C, Lindsey RW (1998). C reactive protein in Orthopaedics. Orthopaedics 21(6):687-691.

Gartner TK, Ogilvie ML (1988). Peptides and monoclonal antibodies which bind to platelet glycoprotein Hb and or HI a inhibit clot retraction. Thromb. Res 49: 43-53.

Heilmann L, Siekmann U (1989). Hemodynamic and hemorhelogical profile in women with proeinuric hypertension of pregnancy and pregnant control. Arch Gynaecol Obstet 246(2):156-186.

Iwaki T, Sandoval-Cooper MJ, Paiva M., Kobayashi T, Ploplis VA, Castellino FJ. (2002). Fibrinogen stabilizes placental-maternal attachment during embryonic development in the mouse . Am J Path 160 (3), 1021-134.

Kametas N, Krampl E, McAuliffee F, Rampling MW, Nicolaides KH (2001). Hemorhelogical adaptation during pregnancy in Latin American population. Eur J Haematol 66:305-311.

Kuijper PH, Gallardo- Torres HI, Lammers JW, Sixma JJ, Koenderman L, Zwaginga JJ (1957). Platelet and fibrin deposition at the damaged vessel wall: cooperative substrate for Neutrophil adhesion underflow conditions. Blood 89, 166-175.

Kupfermine MJ, Eldor A, Steinman N, Many A, Bar-Am JA, Fait G, Lessing JB (1999). Increased frequency of the genetic thrombophilia in women with complications of pregnancy. N. Eng J. Med 340, 9-13.

Lewis SM (2006). Miscellaneous test. In: Lewis, S.M., Brain, B.J.and Bates, I. (2006).Dacie and Lewis practical haematology.10th ed.Churchill Livingstone. Elsevier, Philadelphia, USA.Pp 595-607.

Martin P (1997). Wound healing aiming for perfect skin regeneration. Science 276, 75-81.

McRitchi DI, Girotti M.J, Glynn MJ, Goldberg JM, Rotstein OD (1991). Effect of systemic fibrinogen depletion on intra-alsomine abscess formation. J. Lab. Clin. Med. 118, 48-55.

Pitiphat W, Gilman MW, Joshipura KJ, Williams PC, Douglas OW (2005). Plasma CRP in early pregnancy and preterm delivery. Am J Epi 162(11):1108-1113.

Prisco D, Ciuti G, Falciani M 2005). Haemostatic changes in Normal Pregnancy. Haematological Reports 1(10): 1-2.

Reid HI, Ugwu AC (1987). A simple technique for rapid determination of plasma viscosity. Nigeria Journal of Physiological Sciences.3:45-48.

Robins J, Woodward M, Lowe G, Mccaul P, Cleyne H, Walker JJ (2003). Maternal blood rheology and pregnancy induced hypertension. www.Clin med.netprints.org.

Romero R, Jimmy E, Luis FG, Juan P, Larat F (2001). The role of inflammation and infection in preterm birth. Am. J Obstet Gynaecol 185:1130-1136.

Stirling Y, Woolf L, North WR, Seghatchian MJ, Meade TW (1984). Haemostasis in normal pregnancy. Thromb. Haemost 52, 176-82

Tommasso MD, Fernetti C, Conforti D, D"Acona RL, Cianciulli D, Branconi F (1991). Hematocrit and hemoglobin parameters of hematic viscosity in pregnancy induced hypertension. Minerva Ginecol 43:237-240