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ELEVATION OF HUMAN CHORIONIC GONADOTROPIN HORMONE AND FIBRINOGEN CONCENTRATION AS AN IMMUNOPROTECTIVE MECHANISM DURING PREGNANCY.

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ABSTRACT

This study was aimed at determining the level of serum human chorionic gonadotropin hormones and fibrinogen concentration of pregnant women in Benin City. Subjects aged between 18 and 42 years participated in the study. Patient consent was obtained from 240 pregnant women on antenatal visit and 80 aged matched healthy individual on routine checkup. Serum human chorionic gonadotropin hormones and fibrinogen concentration was estimated using enzyme-linked immunosorbent assays methods. Erythrocyte sedimentation rate was estimated using the westergren method. A significant increase ($P< 0.05$) in serum human chorionic gonadotropin hormones concentration at stage 1 (control group) when compared with stage 3(second trimester), 4(third trimester) and 2(first trimester) and it was also observed that, there was a significant increase ($P< 0.05$) in fibrinogen concentration and Erythrocyte sedimentation rate at stage 1 when compared with stage 2,3 and 4 . The elevation of human chorionic gonadotropin hormone, fibrinogen concentration and erythrocyte sedimentation rate are immuno adaptive mechanism of the blastocyst to get implanted onto the walls of the endomentrium where it can obtain nourishment and prevent any possible rejection by the maternal immune system.

Keywords: Blastocyst; Immune system; Endomentrium; Enzyme-linked immunosorbent assays.

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INTRODUCTION

Human chorionic gonadotropin is a hormone produced by the syncytiotrophoblast, a component of the fertilized egg, after conception (1, 2). It interacts with the luteinizing hormone/choriogonadotropin receptor of the blastocyst and promotes the maintenance of the corpus luteum during the beginning of pregnancy, this allows the corpus luteum to secrete the hormone progesterone during the first trimester, progesterone enriches the uterus with a thick lining of blood vessels and capillaries so that it can sustain the growing fetus(3). HCG due to its highly negative charge can repel the immune cells of the mother thereby protecting the fetus. It has also been hypothesized that hCG may be a placental link for the development of local maternal immunotolerance (3).

Human chorionic gonadotropin also plays a role in cellular differentiation, proliferation and may activate apoptosis (4). Gestational trophoblastic disease like hydatidiform moles ("molar pregnancy") or choriocarcinoma may produce high levels of hCG (due to the presence of syncytiotrophoblast- part of the villi that make up the placenta) despite the absence of an embryo (5, 6, 7).

Fibrinogen is a soluble, 340 kDa plasma glycoprotein, that is converted by thrombin into fibrin during formation, it is synthesized in the liver by the hepatocytes. Fibrinogen, the principal protein of vertebrate blood clotting, is a hexamer, containing two sets of three different chains (α, β, and γ), linked to each other by disulfide bond (8).

Pregnancy-induced hypercoagulability is probably a physiologically adaptive mechanism to prevent *post partum* hemorrhage (9), pregnancy changes the plasma levels of many clotting factors, such as fibrinogen, which can rise up to three times its normal value, Thrombin levels increase (10), protein S, an anticoagulant, decreases. However, protein C and antithrombin III remain constant (11). This study was aimed at determining the level of serum beta human chorionic gonadotropin hormones and fibrinogen concentration of pregnant women in Benin City.

MATERIALS AND 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 by trimester. Those with molar pregnancy were excluded from the study. Ethical approval was obtained from Lahor medical and research laboratory, Benin City, Edo state.

Blood sample collection

6ml of venous bloods were taken from the antecubital vein by venapuncture. It was shared equally into ethylene diamine tetra acetic acid container and an anticoagulant free test tube; allow clotting and subsequently centrifuging at 750xg from 15minutes to obtain serum. The serum was immediately aliquoted into Eppendorf and stored at -20^{0}C.

Blood Sample Processing

Serum human chorionic gonadotropin (hCG) estimation [12]

The hCG ELISA Test is based on a solid phase enzyme-linked immunosorbent assays (ELISA).The assay system utilizes one mouse monoclonal anti-hCG antibody for solid phase (microtitre wells) immobilization and another mouse monoclonal anti-hCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen was added to the hCG antibody coated microtitre wells and incubated with the Zero Buffer (bovine serum, green dye, NaN_3) at room temperature for 30 minutes. The wells were washed with wash solution to remove any residual test specimen and hCG antibody labeled with horseradish peroxidase (conjugate) was added. After incubation at room temperature for 15 minutes, the wells were washed with wash solution to remove unbound-labeled antibodies. A solution of TMB (3, 3', 5, 5' Tetramethylbenzidine) was added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development was stopped with the addition of 1N HCl and measured spectrophotometrically at 450 nm. The concentration of hCG is directly proportional to the color intensity of the test sample(s).

Quantitative fibrinogen concentration estimation method [6]

The principle of the double antibody sandwich ELISA method, in this assay the Fibrinogen present in samples reacts with the anti-Fibrinogen antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-FC antibodies conjugated with horseradish peroxidase (HRP) was added. These enzyme-labeled antibodies form complexes with the previously bound FIB. Following another washing step, the enzyme bound to the microtitre wells was determined by the addition of a chromogenic substrate (3,3',5,5'-tetramethylbenzidine). The quantity of bound enzyme varies directly with the concentration of FC in the sample tested, the absorbance at 450 nm, is a measure of the concentration of FC in the test sample(s).

Erythrocyte sedimentation rate (ESR) estimation [13]

The westergren method requires collecting 2 ml of venous blood into a tube containing 0 .5 ml of sodium citrate. It should be stored no longer than 2 hours at room temperature or 6 hours at 4 °C. The blood was drawn into a westergren-Katz tube to the 200 mm mark. The tube was placed in a rack in a strictly vertical position for 1 hour at room temperature, at which time the distance from the lowest point of the surface meniscus to the upper limit of the red cell sediment was measured. The distance of fall of erythrocytes, expressed as millimeters in 1 hour gives the erythrocyte sedimentation rate.

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Statistical Analysis

All results were presented as mean ± 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 ≤ 0.05 were considered significant.

RESULTS

Table 1: The mean± standard deviation of human chorionic gonadotropin (hCG), fibrinogen concentration (FC) and erythrocyte sedimentation rate (ESR) at stage 1 to 4. It was observed that, there was a significant increase (P< 0.05) in hCG concentration at stage 1 when compared with stage 3, 4 and 2 and it was also observe that ,there was a significant increase (P< 0.05) in FC and ESR at stage 1 when compared with stage 2,3 and 4 .

Table 1: The mean± standard deviation of hCG, FC and ESR of pregnant women at different trimesters and the control group.

	1	2	3	4
Parameters	N = 80	N = 80	N = 80	N= 80
hCG(mIU/ml)	2± 0.06	21000±10^{A}	4200±05^{B}	3900±08^{C}
FC (g/l)	2.5±0.02	3.5±0.03^{A}	4.2±0.02^{B}	5.0±0.01^{C}
ESR (mm/hr)	4±0.01	13±0.01^{A}	16±0.01^{B}	20±0.02^{C}

Keys

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

It was observed that, there was a significant increase (P< 0.05) in hCG concentration at stage 1 when compared with stage 3, 4 and 2.The elevation of hCG in stage 2(first trimester) might be an immuno adaptive mechanism of the blastocyst to get implanted onto the walls of the endomentrium of mother, where it can obtain nourishment and prevent any possible rejection by the maternal immune system. This is line with these findings. HCG due to its highly negative charge can repel the immune cells of the mother thereby protecting the fetus. It has also been hypothesized that hCG may be a placental link for the development of local maternal immunotolerance (14).Human chorionic gonadotropin not only acts as an immunosuppressive agents but also act as a notifying biomarker for pregnancy, thus preventing menstruation by sustaining the function of the corpus luteum(15).

It was also revealed that, there was a significant increased (P< 0.05) in fibrinogen concentration and erythrocyte sedimentation rate at stage 1 when compared with stage 2, 3 and 4; this might be an adaptive mechanism to lower the shear flow in the placental bed during pregnancy and to prevent any possible post partum hemorrhage during childbirth. This is in accordance with these findings. A high level of fibrinogen especially at the third trimester may be beneficial to the mother during delivery to prevent post partum hemorrhage(16).Fibrinogen contributes to both plasma and whole blood viscosity, which enhances red cell aggregation and lower shear circulation in the placental bed during pregnancy. An increase in erythrocyte sedimentation rate is associated with increase in plasma fibrinogen which promotes red blood cell aggregation. (17,18).The initial rise in relative plasma viscosity can be attributed to the increased level of fibrinogen, a major determinant of blood viscosity and flow (19,20,21).Pregnancy is a hypervolaemic state characterized by about 40% increase in plasma volume which invariably results in the reduction in plasma viscosity(16).This is a unique and useful hemorheologic adaptation of pregnancy that prevent 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(16).

Conclusion

The elevation of human chorionic gonadotropin hormone, fibrinogen concentration and erythrocyte sedimentation rate are immuno adaptive mechanism of the blastocyst to get implanted onto the walls of the endomentrium where it can obtain nourishment and prevent any possible rejection by the maternal immune system. The molecular mechanism needs further investigation.

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