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Research Article

Haemorrheological Changes in Malaria Infected Pregnant Subject Attending Antenatal Clinic at Eku Baptist Government Hospital

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Abstract

We assessed the Haemorrheological changes in malaria infected pregnant subjects attending ante-natal clinic at Eku Baptist Government Hospital. Five hundred (500) subjects were used, comprising of 201 randomly selected malaria infected pregnant subjects, 201 randomly selected non malaria infected pregnant subjects (control 1) and 98 randomly selected non-pregnant non-malarial infected subjects (control 2) classified into first, second and third trimesters respectively. Ethical clearance from both the Ethics Committee of the Faculty of Health Science and Technology, Nnamdi Azikiwe University and Eku Baptist Hospital was obtained. The subjects were tested for Packed Cell Volume (PCV), Erythrocyte Sedimentation Rate (ESR), Relative Whole Blood Viscosity (RWBV), Relative Plasma Viscosity (RPV) and fibrinogen. There was a significant decrease ($P<0.05$) in PCV when test subjects was compared with control subjects in their first and second trimester respectively. But there was a non significant decrease ($P>0.05$) in PCV when test subjects was compared with control subjects in third trimester. Also there was significant increase ($P<0.05$) in ESR in the first, second and third trimesters respectively when test subjects was compared with control subjects. Meanwhile the RWBV and plasma fibrinogen showed a non significant ($P>0.05$) decrease in the first, second and third trimester respectively when the test subjects were compared with control subjects. While the RPV showed significant decreased ($p<0.05$) only in the second trimester. From this study, a significant decrease in PCV was identified and also a significant increase in ESR and plasma viscosity in malaria infected subjects was observed, thus indicating alteration in the haemorrheological status.

Keywords: Relative whole blood viscosity; Relative plasma viscosity; Fibrinogen; Pregnancy and malaria

Introduction

Pregnancy is the fertilization and development of one or more offspring, known as an embryo or foetus in a woman's uterus. In pregnancy, there can be multiple gestations [1]. Pregnancy is a sequence of events which results after the fertilization of the ovum by the sperm cell [2]. Child birth usually occurs about 38weeks after conception, in women who have a menstrual cycle length of four weeks, this is approximately 40weeks from the start of the Last Normal Menstrual Period (LNMP) and conception can be achieved through sexual intercourse or In Vitro Fertilization (IVF) treatment. Pregnancy is a unique state where the physiology of a woman is greatly altered to accommodate the newly developing organ "the foetus [3].

Haemorheology on the other hand, is the study of the flow properties of blood and its formed elements including, RBC, WBC, and Platelets) [4]. Haemorheology is a branch of Biorhoelogy that focuses on blood, whereas Biorhoelogy is the branch of Biological sciences that studies the flow and deformation of biological material under the influence of constraints applied to it [5]. Malaria in pregnancy continues to be a serious health risk for pregnant women in the tropics and is associated with increase risk for maternal anaemia. Each year, approximately 50 million women living in malaria endemic countries of which over half of them live in the tropical areas of Africa with intense transmission of *Plasmodium falciparum* [6].

Normal pregnancy is characterised by profound changes in almost every organ system to accommodate the demand of the feta-placental unit and some of the most significant haematological and rheological changes observed includes: Physiological anaemia, Neutrophilia, mild thrombocytopenia, decrease fibrinolytic activity and reduced blood viscosity [7]. According to a previous study [8]. The reduction in peripheral resistance seen in normal pregnancy in order to increase blood flow and facilitate oxygen and nutrient supply to the tissues, is achieved by both peripheral vaso-dilatation and reduction in blood viscosity. So there is need to study all these parameters associated with blood viscosity. Plasma volume increases with gestation to about 40% above pre-pregnancy level at 30weeks followed with a small decrease at terms, while the Red cell mass increases linearly with gestation to about 25% above pre-pregnancy levels [9]. Similarly, total serum protein concentration decreases during pregnancy; it's mainly due to haemo-dilution [8]. Plasma fibrinogen production stimulated by progesterone increases throughout pregnancy [10].

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Blood viscosity, which in high-shear stress is dependent on the interplay of the changes in plasma viscosity and haematocrit, decreases with gestation [11-13]. However, there is controversy as to the change in plasma viscosity, with some studies showing no change with gestation, some reporting an increase, while the others a decrease [8,10]. The importance of the changes of blood viscosity in normal pregnancy has been highlighted by several studies that demonstrated an increase in adverse pregnancy outcome with increasing haemoglobin level (and thus whole-blood viscosity) [8,14]. In his research reported the following as significant haematological changes in pregnancy: Plasma volume increases (45%) relatively with their RBC volume increase (20%) thus: Haemoglobin (Hb) falls to 12 – 13g/l Haematocrit falls to 33-35%, WBC count increases to 8 to $9x10^6$/L due to increase in neutrophils and monocytes. Significant increase in plasma concentration of Factor VII, VIII, IX, and X Fibrinogen increases from 300mg/dl to 450mg/dl at term. Platelet concentration is unchanged or slightly decreased due to haemo-dilution.

Studies have also shown that the rheological properties of blood can be influenced by Packed Cell Volume (PCV), plasma viscosity, red cell aggregation and red cell deformation with plasma viscosity depending on the concentration of plasma protein especially plasma fibrinogen as asserted by [15,16].

This study was therefore aimed at assessing the Haemorrheological changes in malarial infected pregnant subjects attending ante-natal clinic at Eku Baptist Government Hospital.

Materials and Methods

Study site

The study was conducted at the ante-natal clinic of Eku Baptist Hospital, which is a government health facility situated in Ethiope-East Local Government Area, Delta State

Subject recruitment

A total of five hundred (500) age-matched subjects were used for this study, comprising of two hundred and one (201) randomly selected malaria infected pregnant subjects, two hundred and one (201) randomly selected non malaria infected pregnant subjects (control 1) and ninety-eight (98) randomly selected non-pregnant non-malarial infected subjects (control 2). They were classified according to trimester into first, second and third trimesters respectively. Ethical clearance from both the Ethics Committee of the Faculty of Health Science and Technology, Nnamdi Azikiwe University and Eku Baptist Hospital was obtained. Informed consent was obtained from each participant. Other ethical issues such as maintaining confidentiality and avoiding harm were strictly observed. The subjects were tested for Packed Cell Volume (PCV) using micro haematocrit method, Erythrocyte Sedimentation Rate (ESR) using the Westergren method, Relative Whole Blood Viscosity (RWBV) using capillary viscometry (Reid and Ugwu's method), Relative Plasma Viscosity (RPV) using capillary viscometry (Reid and Ugwu's method) and fibrinogen using heat precipitation method.

Sample collection

Six millitres (6.0ml) of venous blood was withdrawn into a disposable plastic syringe from each subject, 3.0ml of it was mixed with 0.3ml of 31.3g/l tri- sodium citrate solution for plasma fibrinogen assay, while 3.0ml of the whole blood was added to di-potassium-ethylene diamine-tetra-acetic acid (EDTA) bottle for the determination of packed cell volume, Whole Blood Viscosity (WBV), Plasma Viscosity (PV) and erythrocyte sedimentation estimation. In the calculation of Relative Plasma Viscosity (RPV) and Relative Whole Blood Viscosity (RWBV), the mean values of the flow rates of whole blood (Tb), plasma ((Tp) and distilled water (Tw) in seconds were applied using the following equations:

$$RPV = Tp\ (s) / Tw\ (s)$$

$$RWBV = Tb\ (s) / Tw\ (s)$$

Statistical evaluation

Data was analyzed using Statistical Package for Social Sciences (SPSS Version 17 Chicago: SPSS Inc.) and expressed as mean ± standard deviation. Student's t test and Analysis of Variance (ANOVA) were used in comparing values between and among mean of groups respectively. P-values less than 0.05 ($p < 0.05$) at 95% confidence limit was considered significant, while P-values greater than 0.05 ($P > 0.05$) at 95% confidence limit was considered as non significant.

Laboratory methods

Diagnosis of malaria: Malaria parasite infection was diagnosed using both stained thick and thin blood film.

Determination of packed cell volume (micro-haematocrit method) [17]: Method: Properly mixed EDTA whole blood was filled into a plain capillary tube to about 2/3rd its length, the free end of the capillary tube was sealed and placed in a micro-haematocrit centrifuge, spun at 12000 rpm for 5minutes and read with the micro-haematocrit reader.

Erythrocyte sedimentation rate (westergren method) [18]: Method: About 1.6ml of EDTA anticoagulated blood was added to 0.4ml of tri-sodium citrate anticoagulant in a small container with a cap and it was properly mixed. A properly calibrated pipette was screwed into the caped container containing the blood and was allowed to stand vertically for 1 hour away from direct sunlight. The value of the ESR was read after exactly 1 hour.

Whole blood viscosity and plasma viscosity (capillary viscometry- reid and ugwu's method) [19]: Method: The whole and plasma viscosity were measured using 1.0ml graduated syringe to which a vertical hypodermic needle (21.6 x 0.8 x 4mm) was fitted. Whole blood and plasma were drawn into the syringe avoiding air bubbles, till the 1.0ml mark was exceeded. The composite syringe with its plunger and needle was held vertically in a retort stand. The plunger was completely withdrawn and immediately the lower meniscus of plasma/ blood fell to the 1.0ml mark, a stop watch was started. This process was repeated twice for each sample. The time required for 1.0ml of whole blood/ plasma to flow down the syringe was noted. The plasma/whole blood viscosity was expressed as Relative Plasma Viscosity (RPV), Relative Whole Blood Viscosity (RWBV) which was the ratio of the flow time for 1.0ml of plasma/ whole blood to the flow time for 1.0ml of distilled water.

Fibrinogen (heat precipitation method) [20]: Method: Fibrinogen was estimated using the heat precipitation method. Two micro-haematocrit tubes about three quarter of their length were

Table 1: Mean ± S.D of parameters compared among malaria infected pregnant subjects, non malaria infected pregnant subjects (control 1) and non pregnant control subjects (control 2).

Subjects	Parameters	PCV (%)	ESR(mm/hr)	RWBV	RPV	Fibrinogen s(g/dl)
Subject 1	Malaria infected Pregnant subject (n =201)	31.09 ±4.32	50.46 ±30.88	3.57 ±24.71	1.98 ±0.46	5.11 ±1.78
Subject 2	Non malaria Infected pregnant Control (n = 201)	33.27 ±2.42	37.69 ±17.81	4.04 ±0.37	2.16 ±0.26	5.06 ±1.37
Subject 3	Non pregnant Subject control (n = 98)	36.34 ±1.56	16.52 ±4.86	4.24 ±0.31	2.26 ±0.20	3.73 ±0.89
	F(P) Value	88.97(0.00)	73.86(0.00)	0.53(0.59)	23.70(0.00)	33.04(0.30)
	Subject 1 vs Subject 2 (P – value)	0.00*	0.00*	0.66	0.00*	0.97
	Subject 1 vs Subject 3 (P - value)	0.00	0.00*	0.73	0.00*	0.00*
	Subject 2 vs Subject 3 (P – value)	0.00*	0.00*	0.00*	0.00*	0.00*

F(P) Value: mean ± SD of parameters compared among malaria infected pregnant subjects, non-malaria infected pregnant subjects and non-pregnant control subjects using ANOVA.
1 vs. 2 (P Value): mean ± SD compared between subject 1 and 2 using Student's t-test.
1 vs. 3 (P Value): mean ± SD compared between subject 1 and 3 using Student's t-test.
2 vs. 3 (P Value): mean ± SD compared between subject 2 and 3 using Student's t-test.

Table 2: Mean ± SD of parameters compared between malaria infected pregnant subjects and non malaria infected pregnant subjects in trimesters.

Parameter PCV (%)	*ESR(mm/hr)*	*RWBV*	*RPV*	*Fibrinogen (g/dL)*
First trimester Pregnant subject MP (n=50) 32.98 ±3.71	45.30 ±3.50	3.50 ±0.94	2.04±0.41	5.49 ±2.06
Pregnant subject No MP 35.04 ±2.24 (n=50)	32.44 ±16.41	4.17 ±0.34	2.22±0.18	5.12 ±1.54
P-Value 0.02*	0.13	1.00	0.06	0.90
Second trimester Pregnant subject MP (n=80) 30.57 ±4.48	51.31 ±12.50	3.78 ±0.73	1.95±0.49	5.00 ±1.65
Pregnant subject No MP 33.39 ±2.1 (n=80)	38.70 ±17.87	z.01 ±0.36	2.16±0.25	5.15 ±1.46
P-Value 0.00*	0.03*	0.11	0.01*	1.00
Third trimester Pregnant subject MP (n=71) 30.35 ±4.20	53.14 ±28.70	3.84 ±0.42	1.99±0.46	4.95 ±1.70
Pregnant subject No MP 31.89 ±1.99 (n=71)	40.27 ±18.15	3.98 ±0.38	2.10±0.31	4.92 ±1.10
P-Value 0.07	0.02*	0.29	0.49	1.00*

MP: Malaria Parasite Infected
P Value: mean ± SD of parameters compared among malaria infected pregnant subjects and non-malaria infected pregnant subjects in different trimesters using students t test.

filled with blood and sealed on one end using bursen flame. It was centrifuged for 5minutes at 12000g, the haematocrit was read and the capillary tube placed inside a water bath at 56 degree Celsius for three minutes and re-centrifuged at 12000g. The thickness of the precipitated fibrinogen was measured under a binocular lens microscope fitted with a calibrated eyepiece micrometer. The fibrinogen concentration (g/l) was estimated as the ratio of fibrinogen precipitate to the height of the plasma plus fibrinogen and multiplied by 100. The mean of the two tubes were taken.

Results

In Table 1 the Packed Cell Volume values was significantly reduced (P<0.05) when comparison between test subjects and control subjects were made. Also Erythrocyte Sedimentation Rate -values were significantly increased (P<0.05) when comparison between test subjects and control subjects (control and control -2) were made. Whereas a no significant increase (p>0.05) was observed in plasma fibrinogen estimation when comparison between test and control

subjects was made. There was also no significant decreased (p>0.05) in relative whole blood viscosity when test subjects was compared with control subjects, while relative plasma viscosity showed a significant decrease (p<0.05) in comparison with the control.

Table 2 shows a significant decrease (P<0.05) in PCV when comparison between test and control subjects in their first and second trimesters were made respectively. But there was no significant decrease (P>0.05) in PCV when comparison between test and control subjects in 3rd Trimester was made. Also there was significant increase (P<0.05) in Erythrocyte Sedimentation Rate in the first, second and third Trimesters respectively when comparison between the test and control subjects was made. Meanwhile the relative whole blood viscosity and relative plasma viscosity and plasma fibrinogen showed no significant (P>0.05) decrease in the in the first, second and third Trimester respectively when the test subjects were compared with control subjects.

Table 3 shows no significant change (P>0.05) in age groups malaria infected pregnant subjects in first trimester in all parameters when age groups (18-25 yrs) was compared to age groups 26-47yrs.

Table 4: shows no significant change (P>0.05) in age groups of malaria infected pregnant subjects in all parameters among the various age groups, when comparison between age groups (18-25 yrs) and age groups (26-47 years) was made.

Table 5: shows that in the third trimester there was no significant change (P>0.05) in all parameter among the age groups, when comparison between malaria infected subject in age groups (18-25 years) and (26-47 years) was made.

Table 3: Mean ± SD of parameters compared between age groups in malaria infected pregnant subjects (first trimester).

Parameters	*n = 18 18 – 25 years*	*n =32 26 -47 year*	*P- values*
PCV	33.44 ±03.35	32.72 ±3.93	0.69
ESR	42.89 ±34.87	46.65 ±31.15	0.39
RWBV	3.82 ±1.05	85 ±6.88	1.00
RPV	2.07 ±0.28	2.02 ±0.47	0.43
FIBRINOGEN	5.26 ±2.42	5.62 ±1.86	0.54

P Value: mean ± SD of parameters compared between age groups in malaria infected pregnant subjects in first trimesters using students t test.

Table 4: Mean ± sd of parameters compared between age groups in malaria infected pregnant subjects (second trimester).

Parameter	*n = 36 18 – 25 years*	*n =44 26 -47 year*	*P- values*
PCV	29.72 ±5.18	31.27 ±3.72	1.51
ESR	62.11 ±36.13	40.84 ±23.56	3.33
RWBV	3.75 ±0.75	3.81 ±0.72	0.35
RPV	1.96 ±0.40	1.93 ±0.55	0.24
FIBRINOGEN	5.33 ±1.67	4.73 ±1.56	1.63

P Value: mean ± SD of parameters compared between age groups in malaria infected pregnant subjects in second trimesters using students t test.

Table 5: Mean ± SD of parameters compared between age groups in malaria infected pregnant subjects (third trimester).

Parameter	*n = 30 18 – 25 years*	*n =41 26 -47 year*	*P- values*
PCV	29.40 ±4.72	31.05 ±3.68	1.59
ESR	63.73 ±32.71	45.39 ±22.81	2.64
RWBV	3.76 ±0.44	3.89 ±0.39	1.33
RPV	2.01 ±0.33	1.97 ±0.53	0.34
FIBRINOGEN	4.89 ±1.46	5.00 ±1.87	0.29

P Value: mean ± SD of parameters compared between age groups in malaria infected pregnant subjects in third trimesters using students t test.

Table 6: Mean ± SD of parameters compared between age group in non malaria infected pregnant subjects (first, second and third trimester).

Parameter	*n = 30 18 – 25 years*	*n =41 26 -47 year*	*P- values*
FIRST TRIMESTER			
PCV	35.35 ±2.46	34.78 ±2.04	0.89
ESR	31.69 ±15.36	33.07 ±17.52	0.29
RWBV	4.15 ±0.38	4.19 ±0.30	0.49
RPV	2.17 ±0.16	2.27 ±0.19	1.98
FIBRINOGEN	4.99 ±1.22	5.28 ±1.78	0.66
SECOND TRIMESTER			
PCV	33.11 ±2.42	33.61 ±1.86	1.02
ESR	36.92 ±17.54	40.16 ±18.02	0.81
RWBV	4.00 ±0.31	4.03 ±0.39	0.43
RPV	2.09 ±0.24	2.22 ±0.25	2.4
FIBRINOGEN	5.22 ±1.17	5.09 ±1.68	0.39
THIRD TRIMESTER			
PCV	32.00 ±2.32	31.77 ±1.53	0.48
ESR	43.44 ±21.42	37.00 ±13.58	1.52
RWBV	3.99 ±0.39	3.96 ±0.37	0.3
RPV	2.14 ±0.26	2.06 ±0.34	1.11
FIBRINOGEN	4.99 ±1.10	4.84 ±1.11	0.58

P Value: mean ± SD of parameters compared between age groups in non-malaria infected pregnant subjects in different trimesters using students t test.

Table 6: shows no significant change in all parameters among the different age groups, when comparison between first trimester non malaria infected pregnant subject in age groups (18-25 years) was compared to those in age groups (26-47 years).

Discussion

Results from previous studies have shown significant changes in the haematological system of pregnant subjects e.g. RBC, WBC, and Platelet and coagulation profile [21,22]. This was confirmed in this study as results revealed a statistically significant decrease in PCV in malaria infected pregnant subjects compared to non malaria infected pregnant subjects (control 1) and non pregnant subjects (control 2). This decrease was with regard to the different age group and gestational period. This was in agreement with earlier studies [13,16]. This reduction could be as a result of the physiological anaemia that pregnant subjects are prone to (due to marked increased in plasma volume without a corresponding increase in RBC) and a further breakdown of subjects PCV by the malaria parasites resulting in severe anaemia [23,24]. This severe anaemia could be as a result of the combined effect of pregnancy and malaria.

Furthermore, a significant increase in ESR was obtained from this study with regard to the different age group and gestational periods, when test subjects were compared with control subjects. This is in line with a previous study of [25,26]. This increase could be due to increased fibrinogen levels produced during pregnancy and anaemia. As ESR is governed by the balance between pro-sedimentation factors, mainly fibrinogen and factors resisting sedimentation e.g. beta potential, high proportion of fibrinogen causes the red cells to stick together to form "rouleaux" which settle faster.

Result from this study also showed that RWBV was reduced, with regard to the different age group and gestational periods, but this reduction was not statistically significant when test subjects were compared to control subjects. This agrees with previous findings [11]. This could be as a result of low PCV concentration, as relative whole blood viscosity correlates with PCV in previous works [11,27].

The RPV was significantly reduced when test subjects were compared to controls, with regard to age groups and gestational period. This however does not agree with some previous study

[16,10], which reported an increased RPV. This could be as a result of malaria burden on pregnancy resulting in pathologic anaemia, which could bring about a further protein changes leading to an alteration in fibrinogen-globin. This agrees with previous study [27].

Furthermore, this study revealed that there was no significant increase in fibrinogen concentration of malaria infected pregnant subjects and non malaria infected pregnant subjects when compared. However, a significant increase (<0.05) was seen in fibrinogen concentration, with regards to age group and gestation, when the pregnant subjects was compared with the non pregnant subjects. This also confirmed previous studies [8,16,22]. This could be as a result of increase protein synthesis to cope with the increase protein needs of the mother and foetus or hormonal changes as levels of oestrogen and progesterone has been reported to increase throughout pregnancy [13].

Conclusion

Result obtained showed a significant decrease in PCV and RPV, when test subjects were compared with controls. A significant increase was also obtained in ESR when the test and control subjects were compared. However, a non significant increase in RWBV and fibrinogen was obtained when comparison between test and control subjects were made. These findings therefore show that there is an alteration in the haemorheological profile of subjects due to severe anaemia, leucocytosis, decreased blood viscosity and thrombocytopenia that these subjects are predisposed to due to malaria in pregnancy, therefore the need for haemorheological examination of pregnant subjects in malaria endemic areas.

Ethical Approval

Ethical approval was sought and obtained from the ethics committee of the Faculty of Health Science and Technology, Nnamdi Azikiwe University, Nnewi Campus and Eku Baptist Hospital, Eku.

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