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EVALUATION OF PROTEIN C, PROTEIN S AND FIBRINOGEN OF PREGNANT WOMEN WITH MALARIA IN OWERRI METROPOLIS

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Abstract

This study was performed to evaluate protein C, protein S and fibrinogen levels in pregnant women with malaria in Owerri Metropolis. The study was carried out at the Owerri, Federal Medical Center in Imo, Nigeria. A total of 600 subjects from 18-45 years of age were recruited for the study. The study included 300 pregnant women with malaria and 300 pregnant women without malaria who visited the Federal Health Center's Owerri, Maternity Hospital. Five milliliters (5 ml) of venous blood was obtained from each participant using standard venipuncture and placed in a simple serum collection container. The results showed an increase in protein C ($P = 0.000$), a decrease in fibrinogen ($P = 0.000$) and no significant difference in protein S ($P = 0.309$) in
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pregnant women with malaria compared with women with malaria. Pregnant women do not have malaria. This study showed that protein C increased, fibrinogen decreased, and protein S remained unchanged. Pregnant women with malaria studied may show changes in these regulatory clotting proteins.

Keywords*: Protein C, Protein S, fibrinogen, Malaria, pregnant women.*

Introduction

Malaria, a parasitic infection caused by the Plasmodium parasite, is a serious public health problem worldwide, especially in underdeveloped countries, causing morbidity and mortality significantly, especially in sub-Saharan Africa, where up to one million deaths per year (Murray et al., 2012; Ogbonna et al., 2021; Ogbonna et al., 2021; Obeagu et al., 2017 ; Okoroiwu et al., 2014). More than 200,000 children are predicted to die each year in sub-Saharan Africa because their mothers contracted malaria during pregnancy (Steketee et al., 2001). Malaria during pregnancy can cause anemia, cerebral malaria, and bleeding, low birth weight for both mother and baby. Regulating useless blood loss and arterial damage, as well as dissolving excessive clots in thromboembolism, is called haemostasis (Obeagu *et al.,* 2022). Pregnancy leads to changes in hemostasis, such as an increase in the majority of coagulation factors, a decrease in the amount of natural anticoagulants, and a decrease in fibrinolytic activity (Bremme, 2003). During normal pregnancy, platelet counts decrease, possibly due to increased destruction and dilution, with a peak in the third trimester (O`Riordan and Higgins, 2003). The aim of the study was to determine the levels of protein C, protein S and fibrinogen in pregnant women infected with malaria in the municipality of Owerri.

Materials and methods

STUDY AREA

This study was carried out in Federal Medical Centre Owerri in Imo State, Nigeria.

STUDY POPULATION AND SAMPLE SIZE

The study enrolled 600 participants between the ages of 18 and 45. The study included 300 pregnant women with malaria and 300 pregnant women without malaria who visited the obstetrics department of Federal Medical Centre, Owerri

The sample size was calculated using the formula of Naing et al., 2006. Pregnant women infected with malaria accounted for 74.6% of the total cases (Ohalete et al., 2011).

$n = z^2 \times P(1-P)/d^2$

Where

n = Sample size

p = prevalence rate 74.6%

z = confidence interval 95% - 1.96

d = Degree of accuracy- 0.05

$N = 1.96^2 \times 0.746(1-0.746)/0.05^2$

= 288

INFORMED CONSENT

Participants were selected from among pregnant women receiving prenatal care. The second group consisted of uninfected pregnant women and uninfected non-pregnant women randomly selected from hospital staff.

INCLUSION CRITERIA

• Pregnant woman with no signs of infection, inflammatory disease or chronic illness.

• Women who are pregnant and have symptoms of malaria.

• Pregnant women between 18 and 45 years old.

• Pregnant women in any trimester

EXCLUSION CRITERIA

Those excluded from the study were:

• Pregnant women with evidence of ongoing contamination such as HIV, tuberculosis and inflammatory diseases;

• Women who have done so no longer give explicit consent;

• Pregnant women in need of urgent care or at risk of pregnancy, including gestational diabetes, preeclampsia and eclampsia;

• Non-pregnant women with evidence of ongoing contamination.

SAMPLE COLLECTION

Five milliliters (5ml) of venous blood was obtained from each contributor using conventional venipuncture methods and poured directly into an undeniable field for serum collection.

LABORATORY PROCEDURES

All reagents were purchased commercially and the manufacturer's standard operating procedures (SOPs) were strictly followed.

A) Malaria Estimation Using Rapid Test kit

As modified by SD BIO LINE One Step Malaria antigen P.F (HRP-II) rapid kit was used.

Procedure

The set is equilibrated at room temperature. I open the test device and label each patient. Samples were collected using the included capillary pipette and transferred to a circular sample well. Four

drops of Test Diluent were added to the dilution well. The kit is placed on a flat bench for 15 minutes before results are obtained.

Malaria Parasite Identification using Giemsa Staining Technique (Cheesbrough, 2005).

Procedure

A drop of blood was placed on the slide to cover the 1520 mm diameter. Blood was spread evenly on the slide to form a thick film, and the slide was air-dried with the slide horizontal. Before staining, Stock Giemsa stains were diluted to a 1:10 dilution using phosphate buffer at pH 7.2. Giemsa stain treatment solution was used to coat a thick film that dried for 30 min, and at the end of the staining period water was used to gently wash the stain off the slides. The blades are briefly rinsed with running water, and the undersides of the blades are dried to remove excess dirt. After air drying in an upright position, observations were made under a microscope using x40 and x100 objectives.

FIBRINOGEN ASSAY

As modified by GIESSE Diagnostics was used.

Procedure

Samples and controls were diluted 1:10 with imidazole buffer (50 µl + 450 µl). 200 µl of the pre-diluted sample was pipetted into a plastic tube and incubated at 37 ° C for 5 minutes. 100 µl bovine thrombin was added and the time to coagulate was recorded.

PROTEIN C ASSAY

Commercial Kit by MELSIN diagnostics was used. Catalogue Number: EKHU-1392.

Procedure

Pipette 50 µl of standard solution into the standard well. Pipette 10 µl of test serum into each sample well. 40 µl Sample Diluent was added to the sample wells. 100 µl HRP conjugation reagent

was added to all wells, stapled and incubated at 37°C for 60 min. Washed 4 times. 50 µl of chromogen solution A and 50 µl of chromogen solution B were added to each well. They were mixed and incubated at 37°C for 15 min. 50 µl of stop solution was added to each well. The optical density of the sample was read with a microliter plate reader at 450 nm for 15 min.

PROTEIN S ASSAY

Commercial Kit by MELSIN diagnostics was used. Catalogue Number: EKHU-1232.

Procedure

Pipette 50 µl of standard solution into the standard well. Pipette 10 µl of test serum into sample wells. 40 µl Sample Diluent was added to the sample wells. 100 µl HRP conjugation reagent was added to all wells, stapled and incubated at 37°C for 60 min. Washed 4 times. 50 µl of chromogen solution A and 50 µl of chromogen solution B were added to each well. They were mixed and incubated at 37°C for 15 min. 50 µl of stop solution was added to each well. The optical density of the sample was read with a microliter plate reader at 450 nm for 15 min.

Statistical analysis

The results were analyzed using SPSS version 20 with the student's test and the value set at $P < 0.05$ is significant.

Results

Table 1: showing mean values of Protein C, Protein S and Fibrinogen of pregnant women with malaria compared to non-malaria pregnant women

Parameters	MP+	Control	t-value	p-value
Protein C	274.46±46.99	2.13±2.73	40.910	0.000^{*}
Protein S	13.35±1.93	15.83±16.88	-1.022	0.309^{NS}
Fibrinogen	8.98±3.10	265.18±45.91	-38.974	0.000^{*}

The table showed increase in Protein C (274.46±46.99, 2.13±2.73, p=0.000), decrease in fibrinogen (8.98±3.10, 265.18±45.91, p=0.000) and no significant difference in Protein S (13.35±1.93, 15.83±16.88, p=0.309) of the pregnant women with malaria compared to non-malaria pregnant women respectively.

Discussion

This study showed an increase in protein C (*P=0.000*), a decrease in fibrinogen (*P=0.000*) and a significant difference in protein S (*P=0.309*) in pregnant women with malaria compared with pregnant women. The fetus does not have malaria. I did it. Protein C and protein S are hemostatic regulators, and fibrinogen is an inflammatory hemostatic protein that is considered one of the responses in the acute phase. Changes were observed in protein C and fibrinogen, and malaria had no effect on protein S. This suggests that in adults, malaria directly affects blood clotting through protein C is more important as an acute regulator of protein S. The reactive phase, fibrinogen, is affected. This process may affect the homeostasis of secondary blood and thus lead to nosocomial outcomes. Pregnant women with malaria should be appropriately monitored to prevent significant interference with these coagulation-regulating systems. Fibrinogen was reported to be increased during pregnancy to a level of 200% before pregnancy (Bremme, 2003), but was decreased in pregnant women with malaria in this study. Malaria may have a negative regulatory effect on fibrinogen in pregnant women with malaria.

The decrease in C and S proteins reported in pregnant women compared with controls was due to a decrease in tPA activity, which remained low until 1 hour after delivery when activity returned to normal. This decrease is due to a gradual, possibly tripling, increase in plasminogen activator inhibitor1 (PAI1) and increased levels of plasminogen activator inhibitor2 (PAI2) (O`Riordan and Higgins, 2003). The placenta produces PAI1 and is the main source of PAI2. The full term PAI2 level is 25 times higher than the normal plasma level (Kruithof et al., 1987). But there was an increase in protein C and no significant change in protein S in this study.

Conclusion

The study showed an increase in protein C and a decrease in fibrinogen and no change in protein S. Malaria in the pregnant women studied may have alterations in these clotting-regulating proteins.

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