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Changes in alkaline phosphatase activity and nutrient contents in *Plasmodium Falciparum* infected cord blood and their relationship to birth weight at term

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ABSTRACT

Placenta alkaline phosphatase is synthesized in the placenta synctiotroblast from the 12th week pregnancy and is probably involved in transplacenta immunoglobulin G (IgG) transport as well as nutrient transport from mother to foetus via cord blood. Pregnant women are highly prone to malaria from p. falciparum. Information on how P. falciparum in cord blood affects alkaline phosphatase (ALP) and cord nutrients among pregnant women in our environment are poorly documented. This study therefore provides information on the influence of P. falciparum infected cord blood on alkaline phosphatase, nutrient levels and birth weight at term. Cord blood samples were collected from 50 cases of uncomplicated normal deliveries at term from the Lagos University Teaching Hospital (LUTH) after obtaining ethical clearance from the Health Research and Ethical committee at the Lagos University Teaching Hospital. The cord blood samples were prepared for the assay of ALP, glucose, cholesterol, total protein and albumin using standard colorimetric methods while the P. falciparum diagnosis was done using the Geisma stain technique. Results show that the P. falciparum infected cord blood had significantly higher ($p<0.05$) ALP (44.21±4.49U/L) activity when compared with the uninfected cord blood samples (26.29 ± 11.54U/L). The uninfected cord blood sample had significantly increased ($p<0.05$) cord nutrients (glucose: 73.09±11.66 mg/dl, cholesterol: 91.44±18.30 mg/dl, total protein: 6.74 ± 0.92mg/dl and albumin: 4.23±0.43mg/dl) when compa red with the infected cord blood nutrients (glucose: 42.92±5.59 mg/dl, cholesterol: 50.03±5.20 mg/dl, total protein: 5.25±0.40 mg/dl and albumin: 3.09±0.24 mg/dl). The ALP levels bear a negative correlation with birth weight (infected cord: 2.17±0.17 kg uninfected 3.59±0.69kg) at term. Evidence from this study show low birth weight as well as low cord blood nutrients in newborn whose cord blood were infected with P. falciparum. The reduced level of cord nutrients in malarial infected cord blood maybe indicative of diversion of ALP to a defense rather than nutrient transfer, hence a compromise in the function of ALP in nutrient transfer from placenta to umbilical cord

Keywords *P. falciparum*, Alkaline phosphatase, Cord blood, Glucose, Placenta.

INTRODUCTION

Placenta alkaline phosphatase or (pALP) is synthesized in the placenta syncytiotrophoblast from the 12th week of pregnancy (1) and is probably involved in trans-placenta immunoglobulin G (lgG) transport (2), as well as nutrient transport from mother to foetus via cord blood (3). Increased synthesis of placenta alkaline phosphatase in cord blood has been observed to correspond to the nutritional demand of foetus during growth. Placenta alkaline phosphatase activity has been reported to contribute to the maintenance of foetal health by mobilizing nutrients (4) and defending the developing foetus from toxic materials (5).

Recently the use of a more sensitive polymerase chain reaction (PCR) assays has routinely identified *falciparum* malaria parasites in 10%–32% of cord blood samples obtained from individuals in areas where malaria is endemic (6 ,7, 8, 9), suggesting that the presence of malaria parasites in cord blood occurs with greater frequency than was previously appreciated. This finding suggests that the presence of malaria parasites may arise from admixture of maternal blood with cord blood at birth and not in utero (10).The problem has long been neglected, but new approaches and commitment offer hope for reducing the burden of malaria in pregnancy and improving the health of mothers and newborns. While the average adult citizen of an endemic region possesses some immunity to the parasite (11), pregnancy causes complications that leave the woman and foetus extremely vulnerable (12). The parasite interferes with transmission of vital substances through the foetal placenta (13), often resulting in stillbirth, spontaneous abortion, or dangerously low birth weight (12).

Numerous studies have established associations between reduced birth weight and increased risk of coronary heart disease, diabetes, hypertension and stroke in adulthood (14, 15). Malaria remains endemic in Nigeria and is more common among pregnant women, with prevalence ranging from 20% to 44%. It leads to significant consequences for maternal and infant health, such as maternal anaemia, responsible for 11% of maternal deaths, and low birth weight (LBW), responsible for 5-12% of all LBW, 43% of preventable LBW babies and contributes to 75,000-200,000 infant deaths each year in Nigeria (16, 17). Information on the role of alkaline phosphatase in transporting nutrients and maintaining foetal weight via *P. falciparum* infected cord blood is scarce. This study provides data on the influence of *P. falciparum* infected cord blood on alkaline phosphatase, nutrient content and birth weight at term.

MATERIALS AND METHODS

Subjects: Fifty uncomplicated normal deliveries at term (19 with *P. falciparum* infected cord blood and 31 without *P. falciparum* infected cord blood) were randomly selected from Lagos University Teaching Hospital (LUTH) after obtaining informed consent from apparently healthy mothers who were between the ages of 24 and 44 years.

Cord blood samples: Blood samples (2ml) were collected from umbilical cord vein into EDTA, Fluoride oxalate and lithium heparin bottles. The samples collected in the fluoride oxalate and lithium heparin bottles were centrifuged at 1200rpm for 5mins at room temperature (28-31°C). The supernatant from the lithium heparin bottle was divided into two portions and stored frozen in bijou bottles until needed for analysis, which was done within 48 hours. Blood sample in the EDTA bottle was used for parasitological examination.

Sample analysis

P.falciparum **diagnosis:** Thick and thin blood films were prepared immediately using the blood sample in the EDTA bottle on the same slide. For thick film, 12microlitre of blood was spread over a diameter of 15mm, while 2microlitre of blood was used for thin films. The thin film was then fixed in absolute methanol for 1-2 seconds and air-dried. The blood film was then stained after 24-48 hrs with 3% Giemsa stain solution at pH 7.2. After which the slides were viewed under the microscope and malarial parasites counted per high power field and the density was graded as follows: 1 parasite/field: Low density (+), 2-9 parasites / field: medium density (++), >20 parasites/field: High density (+++) (18).

Nutrients estimation:

One portion of the serum sample from the lithium heparin was used for the estimation of nutrients (glucose, cholesterol, total protein and albumin) which were determined spectrophotometrically using diagnostic kits (Randox Laboratories Limited, England) by the methods of (19) for glucose and cholesterol and (20) for total protein and albumin.

Alkaline phosphatase assay: The second portion of the lithium heparin serum sample was heated at 60°C for7min to inactivate the other isoform of alkaline phosphatase, leaving only the placental isoform, which is heat-stable. The pALP activity in the heated sample was assayed by the paranitrophenylphosphate, (pNPP) method as previously described (21).

Statistical analysis: All data were presented as Mean± SD, and values compared with the Student's t-Test. Significance level was set at $P<0.05$.

RESULTS

The influence of *P. falciparum* infectedcord blood on alkaline phosphatase and birth weight was investigated and results are presented in Table 1.

Table1 shows the relation between alkaline phosphatase and nutrients in *P. falciparum*infected and uninfected cord blood.

Table 1:Relationship Between ALP and Nutrients in both parasitized and non-parasitized Cord Blood andtheir association with birth weight

	P. falciparum infected cord blood (n=19)	uninfected cord blood (n=31)
ALP (U/L)	44.21±4.49 *	26.29 ± 11.54
Glucose (mg/dL)	42.92±5.59**	73.09±11.66
Cholesterol (mg/dL)	50.03±5.20 **	91.44±18.30
Total protein (mg/dL)	5.25±0.40	6.74± 0.92
Albumin (mg/dL)	3.09±0.24	4.23±0.43
Birth weight (kg)	2.17±0.17*	3.59±0.69

Values are written as Mean ±SD for 'n' numbers
Significantly (p<0.05) higher or lower** than the value obtained from the P. falciparum uninfected cord blood sample.*
ALP= heat-stable alkaline phosphatase

From the results (Table 1), the ALP was higher in the *P. falciparum* infected cord blood compared with the uninfected cord blood, while the cord nutrients (glucose, cholesterol, total protein and albumin) and birth weight were higher in the uninfected cord blood compared with the infected cord blood.

DISCUSSION

Present data (Table 1) show significant increase (P<0.05) in serum alkaline phosphatase in malarial infected cord blood when compared with cord blood not infected by malaria. The increase in ALP in parasitized cord blood could be as a result of the defensive role ALP offers to the umbilical cord, helping to fight against infection and toxic materials (5). ALP increases following polymorphonucleated (PMN) cells activation, which occurs during infections or after stimulation by granulocyte colony-stimulating factor (22). It is therefore possible that PMN have a role in defence against malaria (23). This could also explain the mechanism of ALP as a defense in umbilical cord *P. falciparum* infection. The result showed that cord nutrients (glucose, cholesterol, total protein and albumin) were higher in non- malarial infected blood compared with cord blood infected with *P. falciparum* and these differences were statistically significant (P<0.05) for the four nutrients. It was also observed that the higher the ALP, the lower the cord nutrients with respect to *P. falciparum* infection. The umbilical cord plays a vital role in the transportation of nutrients from the mother to the developing foetus (3). Placental alkaline phosphatase is involved in facilitating the transfer of nutrients across cell membrane of the developing foetus. The cord nutrients being lower in malarial infected cord blood may indicate diversion in ALP activity to a defensive role rather than nutrient transfer, hence a compromise in the function of ALP in nutrient transfer from placenta to umbilical cord (3). ALP level correlates negatively(r= -0.88) with birth weight as it was observed that the higher the ALP, the lower the birth weight. Malarial infected cord blood with higher ALP level had babies with lower weight and the difference was statistically significant when compared with that of uninfected cord blood and this corresponds to the report of (24) which shows that elevation of placental alkaline phosphatase activity may be a useful indicator during the second trimester of pregnancy for detecting the risk of low birth weight. The decrease in birth weight may be as a result of the placental alkaline phosphatase acting primarily in defense (25) rather than transporting nutrients to the growing foetus (3). Also, the results obtained showed that babies with low birth weight had lower cord nutrients (Table 1). Low cord nutrients which could implicate foetal malnutrition have been shown to have adverse neurologic effects in experimental animals (26).

Perinatal problems such as hypoglycaemia, asphyxia, and/or central nervous system sequelae are known to occur primarily in babies with foetal malnutrition (FM), that is, low glucose, cholesterol, total protein and albumin whether appropriate for gestational age (AGA) or small for gestational age(SGA) but not among those who are simply SGA but not malnourished (27). Nutrients pass from placental to umbilical cord and depression of placental

function as an organ of foetal nutrition has been reported (28). It is known that mean birth weight of infants born with infected placentae are depressed (29). Hence infants having low birth weight due to their cord blood infected with *P. falciparum* should be properly monitored to prevent infant mortality. Malaria has been reported to be a cause of high infant mortality, challenging umbilical cord alkaline phosphatase activity. Evidence from this study show low birth weight as well as low cord blood nutrients in newborn whose cord blood are infected with *P. falciparum*. The myriad consequences of low birth weight and decrease in these cord nutrients are well known including the danger it presents to the child.

CONCLUSION

Malaria in pregnancy is still observed and due to trans-placental transmission, results in congenital malaria which is associated with neonatal mortality. It is therefore recommended that pregnant women should be well educated on malaria and the associated health risks. They should be advised on the use of insecticide treated nets and other control measures in order to reduce the risk of being infected.

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