

Hematological Parameters of Neonatal Umbilical Cord Blood among Sudanese Mothers Infected with Malaria Parasite

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Abstract- Background: Malaria is a major public health problem in Sudan and associated with a lot of complications to pregnant women and their fetus leading to alter organogenesis and fetal development. So this study is addressed this problem by examining fetal hematological status in relation to maternal malaria.

Methods: This is a prospective cross sectional hospital based study, conducted in Eldweim teaching hospital, Sudan, Following informed consent, cord blood was taken and fetal hematological parameters were examined in cord blood of 150 malaria infected and 30 uninfected women, using Sysmex and Hemoglobin capillary electrophoresis.

Results: A total of 180 pregnant women were enrolled for this study of which 150 (83%) had peripheral malaria infections detected by thick film and 30 (17%) without peripheral malaria infections. Maternal malaria was associated with increased risk of LBW in infants born to mothers with peripheral parasitaemia (22.7%) compared to infants from uninfected mothers (6.7%). In current study, found an increased in RCDW-SD and HbA2 with p. value 0.007, 0.01, respectively. Our results also, revealed a reduction in cord blood Hb, RBCs and fetal hemoglobin (Hb F) among babies born by malaria- infected women compared to uninfected women, although the difference was not statistically significant p. value > 0.05 while, PCV, MCV, MCH, MCHC, WBCs and platelet did not differ between babies born by malaria-infected women in compared to control one.

Conclusion: Our data showed increased risk of LBW and alterations in RCDW-SD and decrease in cord hemoglobin in malaria-infected women in compared to control subjects. We recommend that further studies be conducted to fully comprehend the association between maternal malaria and its effect on fetal development.

Index Terms- Malaria during pregnancy; pregnant women; birth weight; intrauterine growth restriction; foetal anaemia; cord blood hemoglobin; pregnancy outcome.

I. INTRODUCTION

Malaria during pregnancy is a major public health problem and it is associated with maternal and infant morbidity and mortality in malaria-endemic countries (Joel, 2016). Over 125 million pregnancies are at risk of malaria infection every year (Dellicour *et al.*, 2010), resulting in 10,000 maternal and 100,000-200,000 infant deaths annually (WHO; Desai *et al.*, 2007).

Furthermore, in areas of low malaria transmission and limited pre-existing immunity, pregnant women may develop severe malaria (SM) (Desai *et al.*, 2007), which is often presented as severe anaemia (SA), hypoglycaemia, cerebral malaria (CM) and multi-organ failure, and can be life threatening to both the mother and the fetus (Adam *et al.*, 2005; Taylor *et al.*, 2012).

However, malaria during pregnancy may cause maternal anaemia, which itself can have deleterious effects on fetal development. Maternal undernutrition is also common among pregnant women in these settings, and undernourished women are more likely to have growth-restricted fetuses and babies with reduced birth weight (Unger *et al.*, 2016). To date, it remains unclear whether the susceptibility to, and the impact of malarial infection are affected by maternal nutritional status (Unger *et al.*, 2016).

Additionally, malaria in pregnancy has been associated with significant degree of intrauterine growth restriction, 36% of preterm deliveries, 30% of preventable low birth weight deliveries, 14% of low birth weight deliveries and 15% of maternal anemia (Steketee *et al.*, 2013). Consequently, The effects of pregnancy-associated malaria (PAM) on infants include stillbirth, congenital malaria, foetal anaemia, and low birth weight (LBW), caused by intra-uterine growth retardation (IUGR) and pre-term delivery (Moya-Alvarez *et al.*, 2014). Importantly, Adam and his colleagues investigated the co-epidemiology of malaria and anaemia and their combined impact on maternal and prenatal outcomes in the different regions of Sudan.

The study shows 13.7% of antenatal attendants in New Halfa, Sudan had peripherally microscopically detected *Plasmodium falciparum* malaria, placental malaria (using histological examinations) was prevalent in 32.0-40% and 19.5% of parturient women in New Halfa and Gadarif Hospitals, respectively. Malaria was a risk factor for anaemia in New Halfa and for stillbirths in Omdurman Maternity Hospital (Adam *et al.*, 2011).

Furthermore, malaria is thought to play a role in reduced cord blood hemoglobin concentration or fetal anemia through a combination of systemic and local effects. Systemic effects may be mediated through malaria-induced maternal anemia and local effects through placental malaria infection. While there is currently no agreement as to the main mechanisms mediating malaria-associated fetal anemia, severe or chronic infection and the associated cellular immune responses may result in the consumption of glucose and oxygen that would have gone to the fetus. Histopathologic studies of malaria-infected placenta have also found thickening of the cytotrophoblastic membranes which may interfere with nutrient transport to the fetus, subsequently leading to fetal anemia (Laar *et al.*, 2013).

The contributory role of maternal malaria infection in fetal anemia has been evaluated in a number of studies with varying results. A Malawian study found a higher prevalence of fetal anemia to be associated with increasing peripheral and placental parasite densities (Laar *et al.*, 2013). Other studies as done by Rumi *et al.*, 2015 have found statistically significant connection between maternal anemia and fetal hemoglobin. Also, Adam *et al.*, 2007 demonstrated that maternal anaemia is a risk factor for fetal anaemia. Relatively higher prevalence of fetal anemia was observed among babies born to malaria infected women, HIV-infected women and anemic women even though the differences were not statistically significant (Laar *et al.*, 2013).

In Sudan, women who become infected with *Plasmodium falciparum* during pregnancy have an increased risk of delivering low birth weight babies, abortion, stillbirth and maternal anaemia (Adam *et al.*, 2005). Accordingly, there is significant interest in understanding how malaria influences fetal development and pregnancy outcome among Sudanese mothers.

II. MATERIAL AND METHOD

Study Design and Subject

This is a prospective cross sectional hospital based study design that involve sample collection from 180 pregnant women who have live birth (either normal delivery or via C/S), the study conducted in Eldweim teaching hospital, Sudan. Permission and informed consent was taken in advance from mothers of each newborn or legal guardian, before enrolling any subject. Ethical approval was obtained from University of Khartoum ethical committee.

Inclusion criteria

Women who were infected with malaria parasite during second and third trimesters of their pregnancy were included in the study.

Exclusion criteria

Excluded from the study women with HIV positive or had hepatitis B, C infection, as well as those with multiple

pregnancy, eclampsia or chronic disease such as liver, heart, kidney, hypertension and diabetes.

Collection of Blood Samples

A needle was inserted into the umbilical vein above the clamp and three milliliters of cord blood was collected in to K₂EDTA vacutainers, at the time of delivery for parasitological and haematological examination. Informative demographic data was collected with a predesigned questionnaire giving information about mother age, tribe, type of delivery as well as newborn sex and weight was recorded.

Parasitological Examination

Maternal and cord blood were examined for malaria parasite using microscope Thick and thin blood films were stained by Giemsa (10%) for ten minutes, and then washed in buffer water and left to dry and examined under oil objective for malaria parasite stage.

Haematological Examination

Complete Blood Count (CBC), haemoglobin estimation, platelet and lymphocyte were determined using an automated full counter (Auto Hematology Analyzer (BC-3000 Plus)), as well as, Hb F, Hb A and HbA₂ were estimated using hemoglobin capillary electrophoresis (SEBIA hemoglobin capillary electrophoresis).

Statistical Analysis

Data of the study were analysis using SPSS for proportion, frequency, mean and standard deviations. A P- value of P < 0.05 was used as indicator of statistical significance.

III. RESULTS

One hundred and fifty patients with clinical and laboratory evidence of malaria infection and thirty without peripheral malaria infections seen at Dr Eltaib Mohammed private clinic and Eldweim teaching hospital, were recruited into study. Table 1 Showed association between demographic and obstetric data of women infected with malaria parasite and control subjects. Our patient's population consisted of 41(23%) primigravidae and 139(77%) multigravidae, of these, 21(12%) were normally delivered and 159(88%) were delivered by C/S. Of the 150 pregnant women 43(29%) had malaria infection in second trimester and 107(71%) were infected in third trimester. The risk of LBW was increased in infants born to mothers with peripheral parasitaemia (22.7%) compared to infants from uninfected mothers (6.7%). Table 2 shows haematological parameters comparing between babies born by malaria-infected women and control subjects. The cord blood HBG, RBCs count and HBF were found to be decreased in women infected with malaria (13.97±0.97 g/dl vs. 14.42±0.42 g/dl; 4.05±0.04×10¹²/l vs. 4.18±0.06×10¹²/l; 75.19±0.66% vs. 76.17±1.46% respectively) compared to uninfected women, although the difference was not statistically significant (p > 0.05). RCDW-SD was higher among patients (mean 72.22±6.23×fl) compared to control (68.84±5.76×fl) with p value = .007. Similarly, HbA₂ was also significantly higher among patients (mean .52 ±.23 %) compared

to control (mean $.32 \pm .20$ %) with a significant p value= .011. Mean WBCs count were $11.55 \pm 3.77 \times 10^9/l$ and $11.85 \pm 4.13 \times 10^9/l$ for patients and control respectively with no statistically significant (p > 0.05) also, PCV, MCV, MCH, MCHC, WBCs

count, platelet count and HbA did not differ between babies born by malaria-infected women in compared to control subjects.

Table 1: Frequency distribution of demographic data among infected and uninfected wemen

		Malaria-infected n=150		Malaria-uninfected n=30		P-value of independence test using chi-square
		Count	Column N %	Count	Column N %	
Parity	Primigravidae	32	21.3%	6	20.0%	.870
	Multigravida	118	78.7%	24	80.0%	
Delivery	Labor	18	12.0%	3	10.0%	1.000
	Section	132	88.0%	27	90.0%	
Baby gender	Male	74	49.3%	15	50.0%	.947
	Female	76	50.7%	15	50.0%	
Baby Weight	Low	34	22.7%	2	6.7%	.064 OR=4.1
	Normal	116	77.3%	28	93.3%	
Trimester	In 2 nd trimester	43	47%			
	In 3 rd trimester	107	71%			

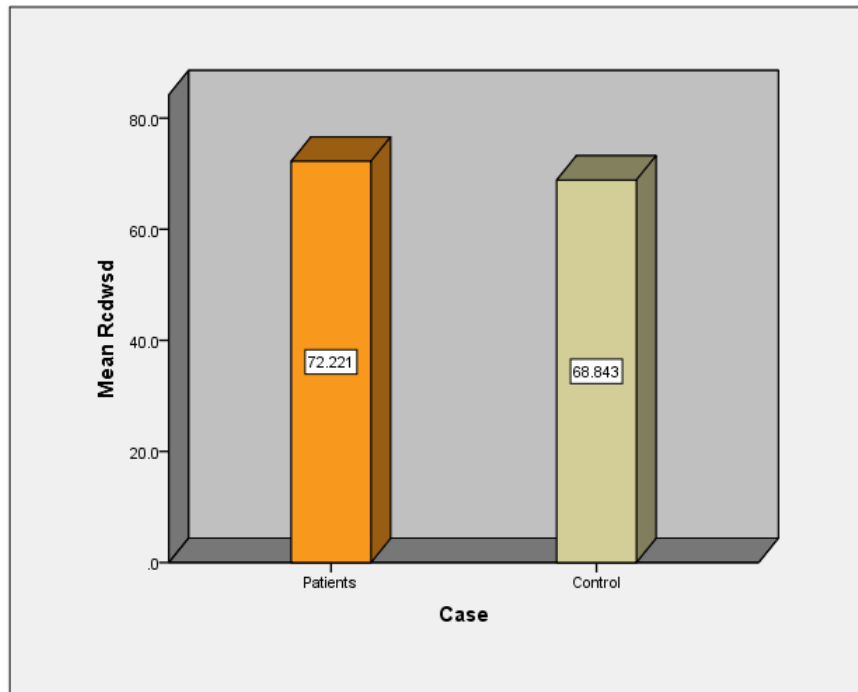
The table Showed that maternal malaria was associated with increased risk of LBW in infants born to mothers with

malaria parasite (22.7%) compared to infants from uninfected mothers (6.7%).

Table 2: Mean ± standard deviation among infected and uninfected wemen

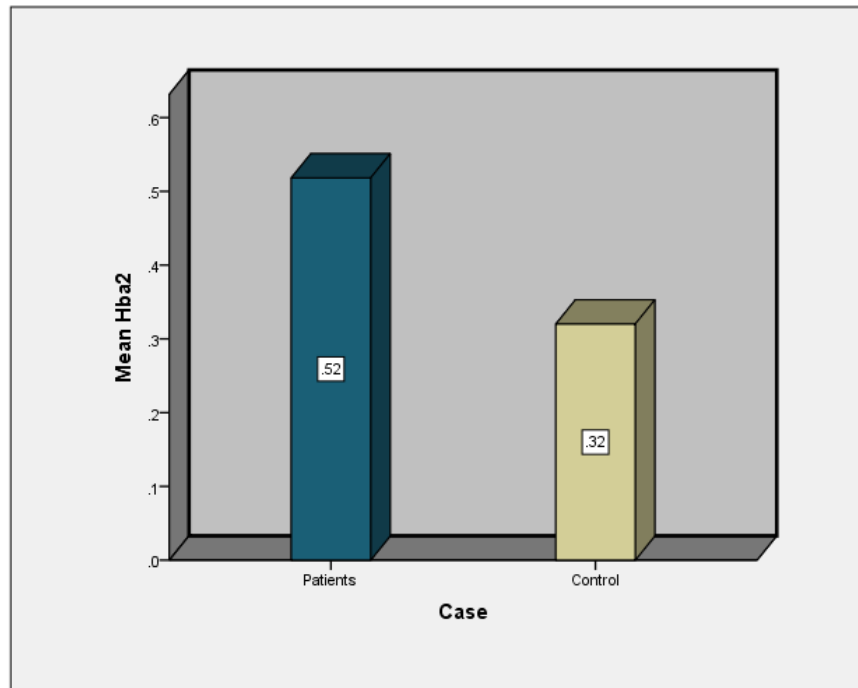
Variables	Malaria-infected n=150	Malaria-uninfected n=30	P-value of independent sample t-test
	Mean ± Std. Deviation	Mean ± Std. Deviation	
Hb (g/dl)	13.97±1.60	14.42±1.08	.140
HbF(%)	75.19 ± 8.04	76.17 ± 7.97	.542
HbA(%)	23.23 ± 7.96	23.67 ± 7.80	.795
HbA2(%)	.52 ±.23	.32 ±.20	.011
PCV(%)	46.28±30.71	45.12±3.37	.837
MCV(fl)	108.40±5.90	108.32±4.37	.943
MCH(pg)	34.54±2.21	34.36±1.43	.674
MCHC(g/dl)	31.88±2.00	31.92±.85	.898
RBCs($10^{12}/l$)	4.05±.47	4.18±.34	.182
RCDW-CV%	17.34±1.71	16.97±1.43	.276
RCDW-SD(fl)	72.22±6.23	68.84±5.76	.007
WBCs($10^9/l$)	11.55±3.77	11.85±4.13	.691
Lymphocyte(%)	43.03±9.76	42.10±11.79	.645
Platelet(%)	245.89±88.42	230.70±76.50	.382

The table showed statistically significant increased in HbA2 and RCDW-SD in infants born to mothers with peripheral malaria parasite (p=0.011, 0.007 respectively) compared to control group.



P-value of two independent groups (.007)

Figure 1. Comparison between descriptive statistics (mean, standard deviation) of RCDW-SD between patient and control groups



P-value of two independent groups (.011)

Figure 2. Comparison between descriptive statistics (mean, standard deviation) of Hba2 between patient and control groups

IV. DISCUSSION

Malaria during pregnancy adversely affects development and survival of fetus through low birth weight, maternal anemia and possibly abortion and stillbirth. So this study conducted to understand how malaria influences fetal development and pregnancy outcome via study infant's birth weight and cord blood haematological parameters.

In our study, infants born to mothers with peripheral parasitaemia had increased risk of LBW (22.7%) compared to infants from uninfected mothers (6.7%). Likewise, Beaudrap *et al.* 2013, reported birth weight was reduced in babies of mothers exposed to malaria in pregnancy compared with the malaria-negative group. These findings were contrary to those results obtained from studies conducted in different areas in Sudan. A study conducted in Gadarif hospital demonstrated that placental malaria had no effects on birth weight (Salih *et al.*, 2011). Another study conducted in Medani hospital showed that, placental malaria infections that were positive by histology were not associated with LBW while submicroscopic malaria infections (diagnosed by PCR) were significantly associated with LBW (Mohammed *et al.*, 2013). Moreover, a study conducted in Rwanda by Rulisa *et al.* 2009, reported that newborn's birth weight was not directly influenced by maternal malaria.

Interestingly, in this study, a significant haematological alteration in RCDW-SD was detected (72.22 ± 0.5 vs. 68.84 ± 0.26 ; $p = 0.007$), no surprisingly, anemia due to inadequate iron supply or folate lead to anisocytosis where the erythrocytes produced are smaller than average size and having a large size variation (Sazawal *et al.*, 2014). additionally, poor nutrition, which contributes to inadequate intake of iron, folate and other micronutrients, is common in the geographic areas where these parasitic infections are prevalent, and may have an important role in the relationship of infections and anemia (McClure *et al.*, 2014), .

In current study, cord blood HBG, RBCs count and HbF were found to be decreased in women infected with malaria (13.97 ± 0.97 vs. 14.42 ± 0.42 ; 4.05 ± 0.04 vs. 4.18 ± 0.06 ; 75.19 ± 0.66 vs. 76.17 ± 1.46 respectively) compared to uninfected women, although the difference was not statistically significant, this finding is similar to the observation of Uneke, et al, 2008, we observed the prevalence of fetal anemia was higher among babies born by malaria infected mothers compared to those of the uninfected mothers, although the difference was not statistically significant ($P > 0.05$). Furthermore, in most parts of the sub-Saharan Africa where malaria is endemic, cord hemoglobin levels have been described as lower-than-expected, and it was hypothesized to result from fetal immune activation to maternal malarial antigens (Uneke, *et al.*, 2008). Recently, Rogawski and colleagues observed that malaria during pregnancy, decreases cord Hb and is a risk factor for fetal anemia in Malawi (Rogawski *et al.*, 2012). Another study done by Brabin 1992, suggested that foetal anaemia also resulted from maternal malaria during pregnancy (Abrams *et al.*, 2005). The reverse finding was reported by Santen *et al.*, 2011 we observed no effect of placental malaria infection or maternal anemia on cord blood hemoglobin concentrations. This finding is agreement with a study conducted in Malawi that also found no significant effect of peripheral or placental malaria infection on cord-blood concentrations of hemoglobin (Abrams *et al.*, 2005).

In this study HbA2 was significantly increase in infants born to mothers infected with malaria ($p = 0.01$) compare to control subject, but HbA no effected. To the best of our knowledge, no other data are available on the influence of maternal malaria on neonatal cord blood HbA2 and HbA levels. Other haematological parameters, such as PCV, MCV, MCH, MCHC, WBCs and platelet count showed no significant differences ($P > 0.05$) between two groups.

V. CONCLUSION

In conclusion, maternal malaria is associated with increased risk of LBW and alteration infant's hematological parameters. These studies have suggested that alterations in RCDW-SD and decrease in cord hemoglobin level may be due to an influence of maternal malaria which may adversely affect pregnancy outcome.

VI. RECOMMENDATION

We recommend that further studies be conducted in White Nile State, Sudan to fully comprehend the effects of maternal malaria, fetal anaemia and under-nutrition on pregnancy outcome via study haematological parameters of the neonatal cord blood.

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