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Placental Alkaline Phosphatase Activity in Serum of Some Nigerian Pregnant Women Infected with Malaria

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Abstract Placental alkaline phosphatase, the heat-stable (hsALP) isoform is produced by the placenta and its activity has been associated with cord blood nutrients and proper foetal growth. Transplacental transmission of *P.falciparum* malaria has been reported and this has been observed to cause congenital malaria, anaemia and reduced neonatal birth weight, an evidence of poor growth. Changes in mean corpuscular haemoglobin concentration (MCHC), an index of anaemia and hsALP activity in serum of *P.falciparum* malarial infected pregnant women were therefore investigated. Forty (40) pregnant women (20 infected with *P.falciparum* and 20 uninfected) were selected from Abraka in Delta State, Nigeria. MCHC and hsALP were estimated as previously described. Results show that Malaria infection during pregnancy reduced MCHC value (34.55±2.29 g/dL) but increased hsALP activity value (96.10±12.39 IU/L) when compared with the value from the uninfected pregnant women (MCHC=38.97±2.26 g/dL; hsALP=66.80±7.59 IU/L). The age of subjects and gestational period did not significantly alter the trend of the observed data. Experimental information suggests that malarial infection during pregnancy induces a measure of microcytic anaemia as judged by the MCHC value, and a degree of compromise in placental (membrane) integrity as evidenced by the elevated serum activities of hsALP. hsALP and nutrient levels in umbilical cord blood should be further studied and results correlated with neonatal birth weight in order to strengthen the present observation and improve the understanding of placental functions during malarial infection in pregnancy.

Keywords Heat-stable Alkaline Phosphatase, Mean Corpuscular Haemoglobin Concentration, Plasmodium Falciparum, Anaemia, Malaria, Birth Weight

1. Introduction

Malaria is a mosquito-borne infectious disease of humans caused by eukaryotic protists of the genus *Plasmodium*. In humans, malarial infection is usually caused by *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale, Plasmodium vivax* and the rare *Plasmodium knowlesi*. *P. falciparum* has been shown to be the common cause of the malarial infection and is responsible for about eighty percent (80%) of all malarial cases and ninety percent (90%) of the deaths arising from malaria[1]. The majority of deaths are among young children (1-5 years) in sub-Saharan African [2].

Malarial infection is known to disturb several metabolic and cellular activities. Increased activities of liver enzyme in serum of affected patients have been observed among Nigerians[3]. This observation was attributed to hepatic damage by perhaps the exoerythrocytic form of *P. falciparum* which inhabits the liver. The parasites also destroy red blood cells and induces anaemia as previously reported[4].

Evidence for transplacental trasmission of malarial has been provided[5], and this has been reported to reduce neonatal birth weight, an indication of poor foetal growth. Malarial infection during pregnancy which has been judged to be high among Nigerians[5] could therefore affect placental functions. The placenta is a major organ formed during pregnancy and is known to be involved in several metabolic activities including the synthesis of hsALP which arranges and transports nutrient materials to the developing foetus via the umbilical cord. The activities of hsALP increases as gestation period increases[6], and this has been observed to support proper foetal growth. Transplacental malarial transmission has been documented by[5], but the effect of the transmission on placental functions, especially regarding the ability to secrete hsALP and maintain its cellular concentration has remained scarce in our environment. This present study was therefore undertaken to determine the activity of hsALP in serum of some Nigeria pregnant women infected with *P. falciparum* malaria and results would be correlated with the mean corpuscular haemoglobin concentration, MCHC.

2. Materials and Methods

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2.1. Subjects

Forty consenting pregnant women (20 infected with *P. falciparum* malaria and 20 uninfected) between the ages of 21-38 years and belonging to different gestation period were selected from the Antenatal Clinic, General Hospital, Abraka, Delta State, Nigeria. Five millilitre of fasting venous blood was collected from each subject using sterile hypodemic needle and syringe in a well seated position after about 10min of rest. The collected blood sample was then prepared for the assay of hsALP activity and determination of mean corpuscular haemoglobin concentration, MCHC. Blood sample was prepared for hsALP assay by centrifuging at 1200xg for 8min at 31°C and the serum obtain was placed in a water bath at 65°C for 7min. This treatment denatures other isoforms leaving the placental isotype of ALP which is heat-stable.

2.2. Assays

Activity of hsALP was determined using the sodium thymonpthalin monophosphate method as earlier described [7]. The commercial reagent's kit was supply by Randox Laboratories, Ardmore, UK. MCHC was determined by the automated haematocrit analyzer using blood sample. The presence or absence of *P. falciparum* infection was confirmed by the thin and thick Giemsa stain in addition to the signs and symptoms.

2.3. Statistics

The data were compared with the Student's *t*-Test, Analysis of Variance (ANOVA) and Duncan Multiple Range Test was used to compare the group means. Level of significance was set at $P<0.05$ using the SPSS package version 16.

3. Results

Serum MCHC (mean corpuscular hemoglobin concentration) and the proportion of hsALP (heat-stable alkaline phosphatase) were estimated among pregnant women with and without *P. falciparum* malarial infection. The results obtained are presented in Tables 1-3.

Table 1. Serum MCHC and hsALP values for malarial and non–malarial infected pregnant Women

	Pregnant women with malaria (n=20)	Pregnant women without malaria (n=20)
PCV (%)	30.34±2.36	32.11±3.49
Hb (g/dL)	10.47±0.95	10.83±1.43
MCHC (g/dL)	34.55±2.95	38.97±2.26
hsALP (IU/L)	96.10±12.39	66.80±7.59

Values are expressed as Mean ± SD for "n" subjects

Values of the haematological parameters (PCV, Hb and MCHC) were reduced among pregnant women with malarial infection when compared with pregnant women without the infection. However, malarial infection during pregnancy increased heat–stable (the placental isoform) alkaline phosphatase activity when compared with the activity value obtained from the pregnant women without malarial infection. These changes were not statistically different ($P > 0.05$).

Table 2. Age differences in MCHC and hsALP activity in serum of pregnant women infected or uninfected with malaria

Age group (yr)	PCV (%)	Hb(g/dL)	MCHC(g/dL)	HsALP(IU/L)
Pregnant women with malarial infection				
15-24(n=6)	30.87±2.26	10.52±0.86	34.00±0.56	96.17±13.04
25-34(n=11)	30.21±2.48	10.50±0.97	34.10±0.85	96.55±13.51
35–44(n=3)	29.75±7.02	10.23±0.98	34.34±12.71	96.83±3.098
Pregnant women without malarial infection				
15-24(n=3)	29.74±1.96	9.37±1.33	31.40±2.77	60.67±1.70
25-34(n=13)	32.12±4.03	11.14±1.40	34.68±1.81	69.63±8.52
35-44(n=44)	33.45±1.62	11.18±0.96	38.27±1.76	70.75±3.56

values are expressed as Mean ±SD for "n" subjects

Table 3. MCHC and hsALP values for pregnant women with and without malaria at different gestation periods

Gestation period (wks)	PCV (%)	Hb(g/dL)	MCHC(g/dL)	HsALP(IU/L)
Pregnant women with malarial infection				
15-24(n=9)	30.40±2.16	10.54±0.77	34.67±0.58	95.56±10.50
25-34(n=7)	30.36±2.64	10.47±1.01	34.32±1.09	100.29±13.69
35-44(n=4)	30.30±2.51	10.38±1.32	34.20±1.54	105.75±6.14
Pregnant women without malarial infection				
15-24(n=3)	34.66±2.34	11.74±1.16	33.68±1.44	63.40±2.90
25-34(n=13)	32.62±2.56	11.56±0.94	33.85±3.06	66.39±8.77
35-44(n=4)	32.53±3.38	11.24±1.34	34.00±1.42	72.00±5.32

Values are expressed as Mean ±SD for "n" subjects

Observations (Table 2) show that both MCHC values and hsALP bear positive relationship as the subjects' ages advanced, but the proportion were more marked among the non–malarial infected pregnant women. However, hsALP values were comparatively higher among the pregnant women with malarial infection. Overall, pregnant women within 35 – 44 year age group had the highest MCHC and hsALP values for both malaria and non–malarial infected pregnant women. These differences were not significant (P>0.05)

Among the pregnant women without malarial infection, MCHC and hsALP values progressively increased as pregnancy advanced. For the malarial infected pregnant women, hsALP also increased as gestational period increased, but values were comparatively higher. MCHC values for these subjects were in the reverse order.

4. Discussion

Changes in mean corpuscular haemoglobin concentration (MCHC) and heat–stable (placental) alkaline phosphatase (hsALP) induced by *P.falciparum* infection during pregnancy were investigated. Results (Table 1) show that malarial infection during pregnancy reduces (P>0.05) MCHC and this was associated with increase in hsALP activity when compared with values obtained from the pregnant women without malaria. The age of subjects (Table 2) and gestational period (Table 3) did not significantly (P>0.05) influence the trend of data observed, but among the pregnant women without malarial infection, MCHC and hsALP progressively increased as their age and gestation period also advanced. Pattern was similar among the malarial infected pregnant women, but hsALP values were higher.

There are various types of alkaline phosphatase depending on the source: liver, bone, kidney, placeenta. Liver ALP has been used as marker of hepatic biliary obstruction[7]. Malarial infection has been reported to increase the serum activity of ALP in both humans[3] and experimental animals [8]. Serum ALP activity is therefore, a potential biomarker for the integrity of hepatic drainage system in acute *P.falciparum* malarial infection.

The activity of ALP in serum during pregnancy has been observed to be increased[9]. This observation may not be due to hepatic obstruction, but the contribution of hsALP produced by the placenta. hsALP is synthesized by the placenta and its activity progressively increases as pregnancy advances[9]. This present study (Table 3) supports earlier reports. The role of hsALP during pregnancy includes the arrangement and transport of nutrients to foetus via the umbiical cord, and this maintains the foetus and contributes to its proper development[6].

Transplacental transmission of *P.falciparum* has been reported[5] and this was associated with congenital malaria and reduced birth weight[5], an evidence of poor foetal nutrition and retarded growth. In this present study, it was observed that *P.falciparum* malarial infection during pregnancy increased hsALP in serum and this was associated with reduced MCHC value, an evidence of microcytic (hypochronic) anaemia.

Data suggest compromise in placental (membrane) integrity hence the possibility of transplacental malarial transmission and reduced birth weight previously reported [5]. *P. falciparum* malarial affects the liver and placental alike.

5. Conclusions

In conclusion, the observed increase in hsALP activity induced by *P. falciparum* infection during pregnancy may likely not support the proper growth of the foetus. Therefore, the activity of hsALP in cord blood and its relation to cord blood nutrients and birth weight induced by *P.falciparum* during pregnancy should be further investigated in order to strengthen the present data and further pin point the effect of malarial infection on placenta functions.

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