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Effect of Umbilical Cord Blood Malaria on Nutrient Contents and Free Radical Activity in Day Old Neonates of the Niger-Delta region of Africa

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ABSTRACT

Umbilical cord blood samples from vaginally delivered day old neonates were examined at delivery. Grading was done according to the severity of plasmodium parasitaemia. Results obtained indicate significantly lower ($p<0.05$) plasma glucose levels although there was no significant difference at 5% probability using ANOVA in the packed cell volume of the neonates. Levels of serum total protein, albumin, gamma glutamyl transferase and superoxide dismutase inhibition were significantly higher ($p<0.05$) indicating increased free radical activity. All these changes were found to be parasitaemia-load dependent. Cord plasmodium parasitaemia may be an indicator of severity of nutrient depletion and increased free radical activity in the day old neonate. Prevention and intervention measures for malaria in pregnancy, therefore is a must for both mother and healthcare providers.

Key words: Umbilical Cord, plasmodium, nutrients, free radical.

INTRODUCTION

Malaria occurs in the tropical and subtropical regions below attitudes of 1,500 metres and is therefore a disease of the hot wet eliminates (Alumanah *et. al.,* 2000). Every year, there is approximately 515 million cases of malaria, killing between one or three million people, majority of whom are young children in the sub-Saharan Africa (WHO, 1992).

Humans are infected with the plasmodium protozoan when bitten by an infective female anopheles mosquito vector and the four species which cause this disease include *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale and Plasmodium malariae*_(Graham, 1998). The majority of infections in the sub-Saharan region is caused first, by *Plasmodium falciparum*, the most dangerous of the four human malaria parasites; and second, the most effective malaria vector – the mosquito anopheles *gambiae* which is the most widespread in the sub-Saharan region and the most difficult to control (WHO, 1994).

The umbilical cord develops from the zygote and consists mainly of two arteries and one vein which serve as a means of nutrient exchange between mother and foetus from the placental bed (Guyton and Hall, 2000). Research has shown that malaria parasites are either exchanged though the placenta into the cord between mother and foetus in-utero or through blood 'mix-up' between mother and neonate during clamping of cord on delivery leading to a phenomenon described as cord malaria parasitaemia (Covell, 1950, Bruce – Chwall, 1952). There is evidence of placenta-cord malaria infection of neonates with plasmodium transmitted from mother to child in pregnancy (Covell, 1950).

In pregnancy there is a transient depression of cell – mediated immunity that allows

fetal allograft retention but also interferes with resistance to various infectious diseases (Meeusen *et. al.,* 2001). Furthermore, cellular immune responses to *P. falciparum* antigens are depressed in pregnant women (Riley *et. al.,* 1989, Fievet *et. al.,* 1995).

The umbilical cord transports nutrients which are major classes of food in their monomer units and these include glucose, albumin, proteins, vitamins, minerals etc (Guyton and Hall, 2002). Free radical activity occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases. (Young and Woodside, 2001). Increased production of reactive oxygen species (ROS) e. g superoxide anion and the hydroxyl radical, are produced by activated neutrophils in the host during degradation of haemoglobin in the parasite and are involved in the damage of vascular endothelial lining during a malaria infection (Postma et. al).

Anti oxidants like super oxide dismutase, catalase, gamma glutamyl transferase, vitamin E among others prevent free radical- induced tissue damage either by preventing formation of free radicals, scavenging them or by promoting their decomposition. (Young and Woodside, 2001).

From the foregoing, malaria infection in pregnancy leading to neonatal malaria and its related effects require more in-depth studies in order to lower the morbidity and mortality of neonates in our environment especially in the endemic swampy regions of the Niger Delta in Nigeria.

MATERIAL AND METHODS

Study Centre and Period

This study was carried out in the labour ward of the General Hospital, Sapele, Delta State, Nigeria between August and October, 2008. Clearance was obtained from the hospital's ethical committee and informed consent taken from the parents of each baby.

Subjects

Fifty pregnant women with age range between 20-39 years old, in labour at term were selected at random and tested. Those with chronic illnesses such as could interfere with the levels of the parameters considered were excluded e.g. HIV/AIDS, anaemia, liver cirrhosis, hepatitis, alcoholism, diabetes mellitus and kidney disorders. Ten (10) classified as Group 1/ control had zero /no parasitaemia. Twenty (20) in Group 2 had grade 1(mild) malaria parasitemia, and Twenty (20) in Group 3 had grade 2(moderate) parasitaemia.

Collection of blood specimen

Cord blood samples were obtained by cleaning the cord with 70% alcohol to avoid maternal blood contamination and incised at approximately 15cm from its attachment to placenta with a fresh blade and 5mls of blood collected, putting 2.5mls each into sterile fluoride oxalate and lithium heparinised containers respectively.

Analysis of serum samples

Determination of packed cell volume (PCV)

Plain capillary tubes were then used to collect cord blood samples from the heparinised containers sealed with flame at one end and centrifuged at 1200 x g for five minutes and assessed with haematocrit reader for packed cell volume.

Determination of malaria parasitaemia

Duplicate thick and thin blood smears on slide were made for each sample and giemsa stained to examine the level of plasmodium parasitaemia. Degree of severity of plasmodium parasitaemia being classified as zero for none, grade 1, mild (+), grade 2, moderate(++) for malaria parasite seen; as adapted from Bulmer and others.

Determination of glucose

This was done using the end point enzymatic colorimetric method with the commercial kit supplied by 18, 2^{A} planta. 08390 Montgat – Barcelona, Spain.

Determination of total protein concentration

This was assayed for according to the biuret method with commercial kit supplied by randox laboratories ltd, Co. Antrim United Kingdom.

Determination of albumin

This was done by end point colorimetric

method using the commercial kit procedure of Joaquim Costa, Montgat- Barcelona, Spain.

Estimation of super oxide dismutase (SOD) inhibition

This was determined using the misra and fridovich method.

Gamma Glutamyl transferase estimation

This was assayed using the commercial kit procedure supplied by Joaquim Costa, Montgat – Barcelona, Spain.

Data obtained were analysed using SSPS for windows version 11. Mean and standard error of mean (S.E.M) were determined for the variables. Proportions and percentages were compared using the X^2 test. $p<0.05$ was taken as statistically significant.

RESULTS

Statistical analysis of the data obtained using the analysis of variance (ANOVA) demonstrate significant ($p<0.05$) decrease in the plasma glucose level and hypoglycaemia of the day-old neonates (Table 1.0). Serum albumin and Total serum protein concentration as well as serum glutamyl transferase and SOD inhibition increased ($p<0.05$) as the level of *P. Falciparum* parasitaemia increased in the subject. Packed cell volume, as determined in the various groups show no significant ($p>0.05$) difference.

Table 1: Effect of cord malaria parasitaemia in day old neonates on plasma glucose, PCV, albumin total protein, gamma glutamyl transferase and super oxide dismutase inhibition activities

	No parasitaemia	Mild parasitaemia (+)	Moderate parasitaemia (+)
Glucose (mg/dl)	56.10 ± 9.95[a]	35.60 ± 5.66[b]	24.25 ± 4.95[c]
Total protein(g/dl)	6.69 ± 1.36[c]	7.92 ±1.02[b]	8.68 ± 1.18[a]
Albumin (g/dl)	3.16 ± 0.20[b]	3.53 ± 0.28[b]	4.25 ± 0.48[a]
Gamma glutamyl transferase (µ/l)	5.78 ± 0.67[c]	8.19 ± 2.17[b]	12.00 ± 4.34[a]
SOD inhibition (µ/ml)	27.30 ± 0.43[b]	28.00 ± 0.32[a]	28.66 ± 0.69[a]
PCV (%)	50.60 ± 1.96	50.35 ± 1.79	50.15 ± 2.35

Values are represented in Mean ± SEM
Mean value of the same row with different superscripts differ significantly ($p<0.05$)

DISCUSSION

Plasmodium falciparum infection is the most common type of malaria infection generally in Africa and particularly in the Niger Delta region of Nigeria (WHO, 1992). It is a febrile illness accounting for 300 – 500 million clinical cases with at least one million consequent deaths (mostly children) annually and 90% of such cases occur in Africa (Samba, 1997).

In this study, plasma concentration of glucose decreased significantly ($p<0.05$) as the degree of severity of malaria parasitaemia increased. Table 1.0, this observation agrees with the finding that *Plasmodium falciparum* infection brings about hypoglycaemia (Cornblath et al, 1993, Koh et al 1998).The concentration of serum albumin and total protein increased as the severity of malaria parasitaemia increased. This can be correlated with the finding that albumin and total protein have thiol groups and are regarded as chain breaking antioxidants during oxidative stress generated by free radicals (Young and Woodside, 2001.The concentration of inhibited Super oxide dismutase increased as degree of severity in parasitaemia increased in indication of increased oxidative stress.

Plasmodium merozoites invade the red blood cells (RBCS) in the erythrocyte phase;

thereafter the erythrocyte undergoes rapid and marked deformation (World Medical Association, 1995). This deformation generates oxidative stress which is attributed to increased concentration of gamma glutamyl transferase produced in the Liver (Fervent and Crisanti, 1998). SOD inhibits oxide radicals (O_2^-) leading to marked inhibition (Fridovich, 1972).

It is therefore pertinent at this point to emphasize the inherent danger of hypoglycaemia as the severity of malaria parasitaemia increases. It can also be postulated from this study that since malaria parastaemia indicates an ongoing disease process, it's consequence on the day old neonates can be quite grane depending on on the degree of severity of the parasite load and this could result in increase morbidity and mortality of neonates from the very first day. A higher proportion of babies with fetal malnutrition have moderate/ grade cord blood parasitaemia (Adebami *et. al.,* 2008). The level of the considered nutrient contents and free radical activities in the day-old neonate may not only be used in the diagnosis of *P. falciparum* infection but also as a determinant of the level of parasiteaemia in the neonates RBCs.

The strong association between fetal malnutrition with infected placentae and cord blood malaria parasitaemia may imply that malaria infection is a major factor in the aetiology of fetal malnutrition in Nigeria (Adebami et al 2007).

Malaria infection therefore poses a threat to the life of the pregnant woman as well as the unborn and day-old neonates. Any pregnant woman diagnosed with malaria should promptly be treated and prevention of malaria infection cannot also be overemphasized

In malaria endemic zones malaria parasitaemia should also be a first line investigation in neonates with fever. These postulations may need more clarifications in further supporting studies of this chemical phenomenon.

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