Effects of Maternal Plasmodium Falciparum Malaria, Anemia and HIV Infection on Fetal Hemoglobin Levels in Nigeria

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Abstract

Fetal anemia has been described as an important public health issue because it is one of the major risk factors for infant anemia which is a life threatening condition and an important cause of hospital admission in many developing countries. In this study, apparently healthy pregnant women were enrolled at child birth. Maternal malaria parasite microscopy, hemoglobin concentration (HbC), HIV infection and cord HbC were determined using standard techniques. Prevalence rates of maternal peripheral malaria, HIV infection and maternal anemia (HbC<11g/dl) were 16.0%, 3.1% and 17.2% respectively. Prevalence of fetal anemia (FA) (cord HbC<12.5g/dl) was 65.6%. Babies of malaria infected mothers had a higher proportion of FA (72.2%) compared to those of the uninfected mothers (64.2%). The prevalence of FA was higher among babies born by HIV-positive women (83.3%) than those of HIV-negative women (65.0%), and higher among babies born by anemic women (82.6%) than those of the non-anemic women (63.5%), but differences were not statistically significant (P>0.05). Improving care of malaria and HIV infection as well as anemia control in pregnancy could reduce FA and improve pregnancy outcome.

INTRODUCTION

Despite considerable improvement in maternal and infant health care delivery services in many parts of African, making motherhood safer which is one of several child survival strategies applied through antenatal care continues to be particularly challenging. Malaria during pregnancy has been described as one of the most complex and severe medical challenges facing humanity particularly in sub-Saharan Africa, affecting an estimated 24 million pregnant women [1]. Each year between 75,000 and 200,000 infant deaths are attributed to malaria infection in pregnancy globally [1,2]. It has been estimated that about 90% of all deaths attributable to malaria in the world today occur in the sub-Saharan Africa and this is because majority of infections are caused by Plasmodium falciparum, the most dangerous of the four human malaria parasites [3].

The sub-Saharan Africa remains by far the worst-affected region by the global HIV/AIDS epidemic, with 25.4 million people living with HIV (Just under two thirds, i.e. 64% of all people living with HIV) [4]. In the malaria-endemic regions of the sub-Saharan Africa, the effects of HIV on maternal health have been superimposed on that of malaria [5]. Each year in the sub-region, approximately 25 million women become pregnant and are at increased risk of infection with P. falciparum [5], also the HIV prevalence rates sometimes exceeding 40% have been reported among pregnant women in the sub-region [6]. Approximately one million pregnancies per year are thought to be complicated by coinfection with malaria and HIV in sub-Saharan Africa [4], because the two diseases are known to critically intersect in pregnancy and have serious consequences in pregnant women, their fetuses, and infants [7,8].

Beside malaria and HIV infection, anemia in pregnancy is another major public health problem in Africa and has been described as an important factor associated with increased risk for poor pregnancy outcomes in developing countries [8]. Even though malaria and HIV infection are recognized as major causes of anemia in pregnancy in the sub-region [9,10], it is however pertinent to state that the etiology of anemia in pregnancy in the sub-Saharan Africa is complex and multifactorial, being also caused by an iron- and folate deficient diet and infections such as hookworm, Schistosoma haematobium and Mycobacterium tuberculosis [11].

Although documented evidence abound on the impacts of

maternal malaria, HIV and anemia on pregnancy outcome in the sub-Saharan Africa, there is however paucity of information on their relationship with fetal anemia. Fetal anemia has been described as common in women with chronic moderate-to-severe iron deficiency anemia; also a severe degree of fetal anemia is reported in several areas where malaria in pregnancy is common [12]. Thus the prevalence of infants born with low cord hemoglobin (fetal anemia) is high in areas where malaria and iron deficiency anemia in pregnancy are common as in the sub-Saharan Africa [13], and the high rate of maternal infection with HIV in this region further increases the risk of fetal anemia [14]. Fetal anemia has been described as an important public health issue because it is one of the major risk factors for infant anemia which is a life threatening condition and an important cause of hospital admission in many developing countries [15,16,17].

The objective of this study therefore was to evaluate the effects of maternal malaria, HIV infection and anemia on fetal anemia with the view to providing scientific information that is relevant to policy development and control program implementation as it relates to the overall maternal, fetal and infant wellbeing in Nigeria and other developing tropical nations threaten by both malaria and HIV infection.

MATERIALS AND METHODS

STUDY AREA

This study was conducted in Abakaliki the capital of Ebonyi State in South Eastern Nigeria, from June 2006 to December 2006 at the Ebonyi State University Teaching Hospital (EBSUTH), Abakaliki, which is the largest health facility in this region. The climatic condition of the area is characterized by two distinct seasons, the wet and the dry seasons, the former takes place between April and October, while the latter occurs from November to March. Malaria transmission in the area is perennial but usually at the peak towards the end of the rainy season.

ETHICAL CONSIDERATIONS

The study protocol was approved by Department of Medical Microbiology/Parasitology, Faculty of Clinical Medicine, Ebonyi State University, Abakaliki, Nigeria. Ethical approval was obtained from the Ethical Committee of the EBSUTH, Abakaliki. All work was performed according to the international guidelines for human experimentation in clinical research [18].

STUDY POPULATION/SAMPLING TECHNIQUE

Pregnant women who fulfilled the following study inclusion criteria were enrolled into the study: (i) attended the antenatal clinic at EBSUTH, (ii) had an uncomplicated pregnancy greater than 32 weeks' gestation (based on the fundal height estimation), (iii) reside in Abakaliki or neighbouring local government areas, (iv) had no obvious clinical evidence of malaria (asymptomatic), and (v) had no known underlying chronic illness. Shortly before child birth informed consent was obtained from each participant and about 5ml of the maternal peripheral blood, was obtained from each participant by venepuncture technique into sterile EDTA container for laboratory analysis. Immediately after childbirth, about 5ml of cord blood was obtained technique into sterile EDTA container for laboratory analysis.

LABORATORY ANALYSIS

Each maternal blood sample was analyzed for malaria parasite infection by performing the microscopy of Giemsa-stained thick and thin blood films. The Plus System was used for the determination of parasite density as previously outlined [19]. All the films were double checked blindly by experienced parasitologists and if there were differences an additional assessment was made by another observer, and the average of the two agreeing counts using the Plus System was recorded. Parasitaemia was graded as 1-10 parasites per 100 thick film fields ('+' or 4-40 parasites per mm3), 11-100 parasites per 100 thick film fields ('++' or 41-400 parasites per mm3), 1-10 parasites per single thick film fields ('+++' or 41-400 parasites per mm3) [19].

The HIV Tri Line Test kits, commercially available (Biosystem INC, Vienna Austria) were first used to screen each maternal serum sample which was separated from the blood, to detect antibodies to HIV-1 and HIV-2. Thereafter the HIV-seropositive samples were confirmed by immunoblot analysis using the BIORAD New Lav Blot kits, commercially available (Bio-Rad Novapath Diagnostic Group US, Oxnard CA.). Manufacturer's instructions were strictly followed to determine the sero-status of the samples.

The haemoglobin concentration (HbC) was determined to assess maternal and fetal anemia using the cyanmethaemoglobin method described previously [20]; reading was done using a spectrophotometer (Bayer RA 50). The WHO definition of anemia in pregnancy i.e., haemoglobin concentration Hb<11g/dl [21], and fetal anemia defined by Hb<12.5 g/dl [13,17] were adopted in this

investigation.

All the analysis was done at the Research Laboratories of Departments of Medical Microbiology and Chemical Pathology of Ebonyi State University, Abakaliki.

STATISTICAL ANALYSIS

Percentage prevalence rates were calculated with their respective 95% confidence intervals. Difference between proportions were evaluated using the chi-square tests. Statistical significance were achieved at P<0.05.

RESULTS

A total of 300 women at full pregnancy term, comprising of 89(29.7%) primigravidae and 211(70.3%) multigravidae were studied and of these, 48(16.0%) had malaria parasite in their peripheral blood. P. falciparum was the only species found. Of the 48 women infected by malaria parasite, 1-10 parasites per 100 thick film fields were recorded in 11(22.9) while 11-100 parasites per 100 thick film fields were recorded in the remaining 37(77.1%). A total of 224 women were screened for HIV infection and of these, 7(3.1%) were HIV-positive. The prevalence of anemia (Hb<11g/dl) among the women was 17.2%. Information was available on the babies born by only 226 of the mothers and resulted to 231 babies, comprised of 119(51.5%) males and 112(48.5%) females. Cord blood (fetal) haemoglobin concentration was determined for only 212 babies. The rest could not be determined due to logistic problems at the labor ward of the hospital. The association of fetal haemoglobin concentration with maternal malaria infection, parity and HIV infection is summarized in Table 1.

Figure 1

Table 1: Association of fetal hemoglobin concentration (HbC) with maternal malaria Infection, anemia and HIV infection among women at childbirth in the Abakaliki-Nigeria.

Maternal Parameters	Fetal HbC (g/dl)		Overall Total
	No.(%) <12.5	No.(%) ≥12.5	
Parity			
Primigravidae	33(67.5)	16(32.7)	49
Multigravidae	106(65.0)	57(35.0)	163
Total	139(65.6)	73(84.4)	212
Malaria Infection			
Infected	26(72.2)	10(27.8)	36
Uninfected	113(64.2)	63(35.8)	176
Total	139(65.6)	73(84.4)	212
HbC			
<11g/dl	19(82.6)	4(17.4)	23
≥11g/dl	120(63.5)	69(36.5)	189
Total	139(65.6)	73(84.4)	212
HIV Status			
Positive	5(83.3)	1(16.7)	6
Negative	134(65.5)	72(35.0)	206
Total	139(65.6)	73(84.4)	212

The prevalence of fetal anemia was 65.6%. The primigravidae had slightly higher proportion of babies with fetal anemia (67.5%) than the multigravidae (65.0%), the difference was also not statistically significant (Chi-square =0.09, df=1, P>0.05). Women with peripheral malaria infection had babies with higher proportion of fetal anemia (72.2%) compared with the uninfected women (64.2%), but the difference was not statistically significant (Chi-square =1.97, df=1, P>0.05). Fetal anemia prevalence was higher among babies born by anemic women (82.6%) than those of the non-anemic women (63.5%) but the difference was not statistically significant (Chi-square =3.32, df=1, P<0.05). The prevalence of fetal anemia was higher among babies born by the HIV-positive women (83.3%) than those of the HIV-negative women (65.0%). Statistical analysis showed no significant difference in the trend (Chi-square =0.86, df=1, P>0.05) (Table 1).

DISCUSSION

A high level of fetal anemia (65.6%) defined by cord blood Hb<12.5g/dl, was observed in this study. This was consistent with an earlier report which indicated that the prevalence of fetal anemia was high in developing countries particularly in malarious areas [12]. In two separate studies conducted in

Southern Malawi, a fetal anemia prevalence of 23.4% [13] and 23.3% [17] were recorded, while in Maputo Mozambique, up to 93% of newborns were found to have fetal anemia [21]. These findings were contrary to those obtained from developed countries where it was shown that anemia in newborns is rare, regardless of maternal status [22]. 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 [12]. Thus it was suggested that exposure of the fetus to malaria antigens due to damage of the placental barrier may make the newborn more susceptible to immunologically mediated hemolysis or to dyserythropoiesis [12].

In this study, 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). This was in conformity with findings from a study conducted in Kisumu, Kenya, where children born to mothers with detectable P. falciparum parasitemia on a peripheral blood film at delivery had a lower mean Hb level at birth compared with children born to mothers free of parasitemia at delivery [23]. Similarly in southern Malawi, a higher prevalence of fetal anemia occurred with increasing peripheral P. falciparum parasite density (p=0.03) and geometric mean placental parasite densities were higher in babies with fetal anemia than in those without (3331 vs 2152 parasites/microl, p=0.07). On the contrary, Abrams et al. [24] noted from their study in Blantyre, Malawi, that, though malaria was associated with a reduction in maternal hemoglobin (10.8 g/dL vs. 12.1 g/dL, $p < 0.001$), no reduction in cord hemoglobin and no significant relationship between maternal and cord hemoglobin levels were found. According to their report, cord blood markers of hematological and hypoxic statuses did not differ between malaria-infected and uninfected women. This lack of consistency in the findings from various studies may be explained on the basis of the fact that malaria in pregnancy varies with transmission intensity, access to treatment, coverage and quality of antenatal services, and drug resistance, among others [15-17]. Variations in these factors may account for the differences in the relationship between maternal malaria and fetal anemia. Furthermore, the etiology of fetal anemia is complex and multifactorial and so maternal malaria could either play a major or minor role depending on local epidemiological situation [12].

Maternal anemia was not significantly associated with fetal anemia (P<0.05) in this study, which suggests that maternal anemia at childbirth might not play a central role in the development of fetal anemia. On the contrary, in Southern Malawi, maternal Hb at delivery < 8 g/dl (AOR 1.61, 1.10-2.42) or <11 g/dl (AOR 1.60, 1.10-2.31) was found to be a major factor associated with fetal anemia [13]. And in an assessment of nutritional anemia in pregnant Beninese women and its consequences on the hematological profile of the newborn, haemoglobin concentration was significantly lower in babies born of mothers with Fe-deficient anemia than in babies born of Fe-sufficient mothers [25]. These findings were however, not consistent with the results from a similar study conducted in Blantyre, Malawi, where cord haemoglobin values were not correlated to maternal hemoglobin concentration [24]. The reason for this was not clear but authors noted that the high cord hemoglobin levels observed may relate to the high rates of antimalarial usage and iron supplementation in the Malawian women studied, which might provide a protective effect.

Furthermore it was argued that the maintenance of cord hemoglobin levels despite the presence of maternal anemia appeared to suggest that the fetus has developed mechanisms to preferentially obtain sufficient iron and produce adequate amounts of red cells, since it was long shown that iron is transported unidirectionally from mother to foetus across a concentration gradient [26], and thus stores should be preferentially preserved in the fetus [24]. However, mounting evidence indicates that maternal iron deficiency in pregnancy reduces fetal iron stores, perhaps well into the first year of life and this deserves further exploration because of the tendency of infants to develop iron deficiency anemia and because of the documented adverse consequences of this condition on infant development [27]. Therefore because of a wide range of unanswered questions about the mechanisms involved in the relationship between maternal anemia and fetal anemia, further studies using molecular biological tools are urgently needed to properly elucidate this in the sub-Saharan Africa.

In this present study, the prevalence of fetal anemia was considerably higher among babies born by the HIV-positive mothers compared to those of the HIV-negative mothers. Although there is paucity of published data on the relationship between maternal HIV infection and fetal anemia in sub-Saharan Africa, an available report from Western Kenya, indicated that maternal infection with HIV

was a major risk factor to fetal and infant anemia not only directly, through mother-to-child transmission of HIV, but also indirectly, as suggested by the finding that infant anemia was worse in HIV-uninfected infants when born to HIV-seropositive mothers compared with those born to HIV-seronegative mothers [28].

In conclusion it is imperative to state that a major limitation to this study was our inability to evaluate other potential causes of fetal anemia including blood loss (through obstetrical causes and internal hemorrhage) and red blood cell destruction (through intrinsic and extrinsic causes). This may have affected adequate assessment of the effects of maternal malaria, HIV infection and anemia on the fetal anemia. The inclusion of these factors in future studies is advocated. Another limitation worth mentioning was the use of microscopy technique for the screening of maternal malaria. Although this method is arguably the "gold standard", it is important to note that the possibility of under-diagnosis cannot be completely ruled out. However, because of the public health significance of fetal anemia and other adverse perinatal outcomes associated with maternal malaria, anemia and HIV infection in pregnancy, the importance of effective interventional effort cannot be overstated. Interventions should aim to reduce fetal anemia by improving malaria, HIV infection and anemia control and treatment in pregnancy and by addressing the determinants of pre-term delivery as this may also affect fetal haemoglobin levels [13].

It has been suggested that improving antimalarial control and iron supplementation throughout pregnancy should have direct effects on reducing fetal and infant anemia and improving child development and survival [29]. Antiretroviral regimens can also improve the health of HIV-positive pregnant women and reduce fetal anemia. The WHO guidelines currently recommend highly active antiretroviral therapy (HAART) using the combination of nevirapine, lamivudine,and either stavudine or zidovudine for HIV-infected pregnant women with clinical or laboratory evidence of immunosuppression [30]. There is the need for the integration of the delivery of malaria, anemia and HIV interventions within existing health services in the sub-Saharan Africa, particularly through the antenatal care services as this may permit effective utilization of human resources and address serious resource constraints.

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