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Co-infection of HIV and malaria parasites in pregnant women attending major ante-natal health facilities in Akure, Ondo State, Nigeria

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Malaria and human immunodeficiency virus (HIV) in pregnancy are the major factors contributing to adverse maternal and perinatal outcome. HIV increases pregnant women's chances of contracting malaria, increases the risk of developing anaemia, delivering a low birth weight infant and premature delivery. This study was designed to investigate the level of co-infection of malaria parasite and HIV in pregnant women in State Specialist Hospital, Akure. 616 pregnant women aged 15 to 46 years who attended major referral health facilities for ante-natal purposes between February and April, 2012 were included for the study. 'Determine' and Uni-Gold rapid diagnostic tests kits were used to determine HIV status, giemsa stained thick blood smear of patients were examined for presence of the asexual stages of *Plasmodium* parasite. Out of 616 pregnant women, 28 (4.55%) were HIV positive and 597 (96.92%) had malaria parasite. Among HIV negative women, 569 (96.8%) had malaria parasite infection while 28 (100%) of the HIV positive women had the infection. This study revealed that 92.37% had malaria alone, none of the women had HIV only and 28 (4.55%) were co-infected with both pathogens, indicating that all HIV positive women also harbor malaria parasite. There was moderate correlation between HIV and malaria parasite $p < 0.05$ which is suggestive that women infected with HIV are most likely to be infected with malaria due to their compromised immune system which makes them more susceptible to malaria infection. The rate of malaria infection was generally high in the sampled population though majority was infected at low parasiteamia. This is therefore attributed to the endemic nature of the disease in Ondo state of Nigeria

Key words: Human immunodeficiency virus (HIV), malaria, co-infection HIV-MALARIA, antenatal care, pregnant women.

INTRODUCTION

Human immunodeficiency virus (HIV) and malaria are among the leading causes of morbidity and mortality in sub-Saharan Africa, where they represent common infections in women of childbearing age. Together, they

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cause more than four million deaths per year (World Health Organization (WHO), 2011). About 90% of the 300 to 500 million annual acute episodes of malaria are reported in this region, and there is also an estimated 30 million HIV-infected cases (Rowland-Jones and Lohman, 2002). The burden of malaria is particularly severe in children under 5 years (who constitute more than 70% of the 1 million deaths due to malaria) and pregnant women. About 55% of HIV infected adults in sub-Saharan African are women of reproductive age and they account for over 80% of the world's HIV infected women. In this region, the prevalence of maternal malaria is 65% and HIV affects 40% of the pregnant population (Ned et al., 2005). Schantz-Dunn and Nawal (2009) recorded that malaria is one of the most devastating infectious diseases, killing more than one million people annually. Forty percent of the world's population lives in endemic areas. Malaria epidemics have devastated large populations. Consequently the disease poses a serious barrier to economic progress in many developing countries. There are an estimated 300 to 500 million cases of clinical disease per year, with 1.5 to 2.7 million deaths.

Abu-Raddad et al (2006) estimated that, the interaction of malaria and HIV in one Kenyan district alone had caused 980,000 excess malaria episodes and 8,500 excess HIV infections since the emergence of HIV in the 1980s. In areas endemic for malaria, it is estimated that at least 25% of pregnant women are infected with the disease. The highest risk of infection and morbidity is found among the primigravidas, adolescents and those co-infected with HIV (Desai et al., 2007). Malarial infection in HIV-positive women is associated with higher levels of parasitemia, leading to a greater risk of severe anemia. Likewise, HIV viral load is increased, creating opportunity for infection and more severe disease (Brentlinger et al., 2006). As a result of the impaired immune state in pregnant women, HIV infection increases susceptibility to malaria and the morbidity associated with it in co-infected women, resulting in higher incidences of severe anemia and low-birth-weight neonates in co-infected. WHO (2004) explained co-infection of malaria and HIV having disproportionate effects on pregnant women and pose serious risks. Briand et al. (2009) reported that HIV-infected women were approximately 22 times more likely to die than HIV-uninfected women. There was a similar risk for those with malaria. Women with dual infections thus had the greatest risk of death compared with those with only HIV infection or malaria infection. Malaria infection in HIV-positive pregnant women can also increase the risks of mother to child transmission (MTCT) during pregnancy, labor as well as during breastfeeding period due to increase in the level of HIV in the blood, that is increase in viral load (UNICEF, 2003). Thus any interaction between malaria and HIV can be an additional health burden, especially in pregnant women, with resulting poor birth outcomes and the imminent transmission of these intracellular pathogens. Adeoti et al. (2012) studied prevalence of HIV and malaria parasites co-infection in pregnant mothers and their babies post-delivery, clearly showing that the recruitment profile of the samples examined exhibited prevalence of malaria infections in the HIV infected mothers and their infants. The aim of this research work is to determine the prevalence and the level of co-infection of HIV and malaria infection in pregnant women at Akure, Ondo State, Nigeria using State Specialist Hospital Akure.

MATERIALS AND METHODS

An ethical clearance was sought and collected from the Ondo State Ministry of Health for permission to carry out the present research that involved human subject. The study subjects consisted of 616 pregnant women aged 15 to 46 years, who came from different local government areas, for antenatal purposes at Ondo State Specialist Hospital located in Akure, between February and April, 2012. They were selected randomly without the prior knowledge of their clinical and family history.

Sample collection

Venous blood was collected from the upper arm of the pregnant women with the assistance of trained Laboratory Scientists, following the method described by cheesebrough (2005). 5 ml of blood needed for the tests was drawn gently at site selected, using sterile needle and syringe into the anticoagulant (EDTA) bottles. Each sample bottle was labeled to correspond to the age and number of subject at the point of collection.

Determination of human immunodeficiency virus (HIV) status of pregnant women

Standard commercially sourced 'Determine' and "Uni-Gold rapid diagnostic tests kits were used to determine the HIV status of the subjects. 'Determine' HIV Rapid Test kit (For use with whole blood, serum, or plasma) (www.who.int/diagnostics laboratory). Uni-Gold test kits are kits that pick or react to only HIV in the blood sample (The Trinity Biotech *Uni-Gold*™ HIV test).

"Determine diagnostic test kit" procedure

The expiration date of kit was checked to ensure the kit had not expired, after which the protective wrapper was removed from the test device and the kit labelled with the patient identification number. Disposable pipette was filled with blood sample and held over the sample pad marked by an arrow symbol into which two drops of sample was carefully added. Blood was allowed to absorb into the sample pad and result was read and recorded after waiting for 60 min. Any positive result was subjected to Uni-Gold test to confirm the positivity of the test result.

Table 1. Age distribution of HIV antibodies among pregnant women.

Age group (years)	No. examined	No. positive	Percentage positive
15-19	13	1	7.69
20-24	49	2	4.08
25-29	211	9	4.27
30-34	222	11	4.96
35-39	104	5	4.81
40-44	14	0	0
≥45	3	0	0
Total	616	28	4.55

Uni-Gold diagnostic test kit procedure

The expiration date of kits was checked to ensure they had not expired, after which the protective wrapper was removed from the test device and the kit was labelled with the patient identification number. Disposable pipette was filled with sample and held over the sample pot, into which two drops of sample was carefully added. Two drops of appropriate wash agent was also added to the sample pot. Result was read and recorded after waiting for 20 min for reaction to occur. The appearance of a line in the control region indicates a negative test result while a line in both the test and control regions is indicative of a positive result. When no line appears in the control region, the test was regarded as inconclusive and consequently repeated with a fresh kit.

Malaria diagnosis in pregnant women

All thick films were prepared and stained with Giemsa stain using a modification of method described by Cheesebrough (2005). The approximate numbers of parasites were reported using plus sign: 1 to 10 parasites per 100 high power fields as +, 11 to 100 parasites per 100 high power fields as ++, 1 to 10 parasites in every high power field as +++, and if no parasites were found after examined as NPF (Cheesbrough, 2005). This was done by a trained and experienced laboratory scientist.

Analysis of data

Analysis of data was carried out using MS-Excel and SPSS version 16.0 (SPSS Chicago Inc., IL, and U.S.A). Pearson correlation analysis was used to investigate possible relationship or correlation between HIV and malaria positivity. Statistical significance was set at p value < 0.05.

RESULTS

Prevalence was calculated by comparing the number of positive individuals with the total number examined. Also, for the analysis, the population was grouped into seven age categories and for each age group of pregnant mothers, the number positive and infection rate per category was estimated.

Results of HIV screening in different age groups of pregnant women

Result in Table 1 showed that out of the six hundred and sixteen sampled populations, 28 women representing 4.55% were positive to HIV infection. The prevalence of HIV antibodies occurred most among women aged 15 to 19 (7.69%) followed by those aged 30 to 34 years (4.96%) while none of the women aged 40 years and above was found to be infected by the virus. The distribution of infection among other age groups of women did not follow any particular pattern. For instance, women aged 20 to 24 had (4.08%), 25 to 29 had (4.27%), 30 to 34 had (4.96%), and 35 to 39 years had (4.81%). Result also shows that HIV infection rate decrease as age of women increases, with the exception of mothers aged 25 to 29 and 30 to 34 years, where infection rate increased slightly from 4.27 to 4.96%.

Prevalence of malaria parasitaemia in the different age groups of pregnant women

In Table 2, a total of 597 pregnant women representing 96.92% of those tested had malaria infection. The infection was generally high in the sampled population. Women aged 15 to 19 and 40 years and above were all infected with malaria parasite. The rate of infections increased steadily through the age group, starting from age 20 to 24, with 95.92%, up to age 35 to 39 years, with 99.04%.

Characterization of malaria parasite density in relation to age of positive pregnant women

The density of malaria parasite in the different age groups of the 597 infected pregnant women was categorized as follows: (+) low, (++) moderate and (+++) high parasiteamia. Result indicated that malaria with very high

Table 2. The prevalence of malaria parasite among the different age groups of pregnant women.

Age group (years)	No. examined	No. positive	Percentage positive
15-19	13	13	100
20-24	49	47	95.92
25-29	211	203	96.21
30-34	222	214	96.39
35-39	104	103	99.04
40-44	14	14	100
≥45	3	3	100
Total	616	597	96.92

Table 3. Characterization of malaria parasite density in relation to age of positive pregnant women.

Age group (years)	Malaria parasite density		
	Low (+)	Moderate (++)	High (+++)
15-19	12	1	0
20-24	42	5	0
25-29	187	16	0
30-34	201	12	1
35-39	97	6	0
40-44	14	0	0
≥45	3	0	0
Total	556 (93.17%)	40 (6.7%)	1 (0.17%)

Age long medically accepted mode of classification of malaria parasite density in patients.

high parasiteamia was found only in pregnant women aged 30 to 34 (0.17%), moderate parasiteamia was found in mothers aged 15 to 39 (6.7%) while the older ages were not infected, and low parasiteamia was found in all the age groups (93.17%) (Table 3).

Result of co-infection of HIV and malaria parasites among pregnant women

Result obtained on the co-infections of HIV and malaria parasites in this present study ascertain the proportion of pregnant women carrying concurrent infections of both HIV and malaria. This study revealed 28 (4.55%) positivity to both pathogens, 92.37% had malaria alone and none of the women had HIV only (Table 4). Pearson's correlation coefficient (r) of 0.5 shows moderate correlation. There was a significant correlation ($P < 0.05$) between HIV and malaria parasite infection in the pregnant women (Table 5).

DISCUSSION

The present 4.55% prevalence of HIV antibodies in Akure, Ondo State was not surprising based on the number of AIDS cases reported from Nigeria (WHO, 1995). In addition, personal observation showed that practices identified as high risk factors in the contraction and transmissions of HIV infections (Olusi et al., 1996; Olusi, 1998) are common in the state. Such practices include prostitution, keeping of multiple sex partners, use of unsterilized or partially sterilized needle and syringes by qualified and unqualified health workers, especially in mission houses. As a result of these, the majority of persons in the study group can be assumed to have a history of risky practices.

The differences in the pattern of HIV infection in the age group of pregnant mothers in this study has to do with the known epidemiological features of HIV infection (Olusi et al., 1996). The absence of HIV antibodies among women aged 40 years and above suggested that

Table 4. Prevalence of HIV and Malaria parasite Co-infections among pregnant women in Akure.

Infection	No. examined	No. positive	Percentage positive
HIV and Malaria	616	28	4.55
Malaria alone	616	569	92.37
HIV alone	616	0	0

Table 5. Correlation of malaria parasite and HIV.

Parameter		Value	Asymptotic standard error (a)	Approximation T (b)	Approxinmate significance
Interval by Interval	Pearson's R	0.500	0.074	14.078	0.000 (c)
No. of valid cases		597	-	-	-

[a]Not assuming the null hypothesis. [b]Using the asymptotic standard error assuming the null hypothesis. [c]Based on normal approximation.

most of the women in this age group had formal education about the infection and took extra care by keeping to one sex partner and more so that they are in their late reproductive ages, while other age groups could probably have contracted the HIV antibodies through heterosexual intercourse and carefree attitude, most especially among the young pregnant women aged 15 to 19 years that are still sexually active; these therefore probably indicates the reason for the prevalence and distribution of HIV infection among pregnant women in Akure, Ondo State.

The present work is in contrast to the research work done at Cameroon by Nkuo-Akenji et al. (2011) who observed the prevalence rate of 21.1% HIV infection and a higher number (60.7%) of HIVSP patient in ages 26 to 35 and 14.1, 20.0 and 0% in ages 15 to 25, 36 to 45 and > 45, respectively. Perrault (2009) reported mean HIV prevalence in pregnant women attending antenatal clinics in South Africa, where he noted prevalence rate of 15 to 40% in South Africa but a lesser prevalence of 5 to 10% in East Africa. Adeoti et al. (2012) also exhibited 19 (12.8%) prevalence of HIV infection among 149 pregnant mothers examined.

The result obtained in the present study indicates that 96.92% of the population of pregnant women examined had malaria parasite, though majority (93.17%) of the mothers were infected at low parasitaemia, which is suggestive of the reason why they did not come down with malaria despite being infected. This indicated that pregnant women could be carriers of malaria parasites without showing any symptoms to the infection. The high infection rate in this study therefore is attributed to the endemic nature of the disease in Ondo state of Nigeria.

The results obtained in this work and those of previous studies support the endemic nature as well as in other part of the country. For instance, Martra et al. (1993) found *Plasmodium falciparum* affecting 97.27% cases of pregnant women examined. Falade et al. (2008) found 89% asymptomatic malaria parasite among pregnant women attending antenatal in a Secondary health care facility in Ibadan, Nigeria. Adefioye et al. (2007) also recorded 72% prevalence rate and Chukwurah et al. (2003) recoded 63.5% of prevalence of malaria parasite in pregnant women in Akwa Ibom, Nigeria. The high infection rate observed among women aged 15 to 19 and 40 years and above is probably an indication of low immunity to malaria infection due to primigravidae status of pregnant women (first pregnancy), and so they are more susceptible to malaria infection than others (Martra et al., 1993). Immunity to malaria infection diminishes significantly in pregnancy, particularly in primigravidae (Oforie et al., 2009). In first and second pregnancies, women are especially vulnerable to malaria infection. Mcgregor (1984) identified the factors responsible for susceptibility of primigravidae to malaria as inhibition of type 1 cytokine responses (interferon, interleukin 2 and 12 and TNF). Brabin (1991) also confirmed that the primigravidae were more susceptible to malaria infection than the multigravidae. Opare Addo et al. (2002) reported that primigravidae, alongside those women in their second pregnancy, were more vulnerable to malaria parasitemia. This work is in contrast to the work of McGregor (1983) who reported a decline in malaria prevalence as age increase, as well as with improved host immunity, thus reducing susceptibility in later years.

The percentage of women carrying both infections in the study indicated that all the pregnant women that were positive to HIV antibodies also habour malaria parasites. This research work compared with other recent studies, Adeoti et al. (2012), observed that the prevalence of

malaria infections in HIV infected mothers was due to the impaired immune status of HIV infected pregnant women to control *P. falciparum*. It was also observed that the pregnant women that are infected with the HIV severely suffered from malaria. Previous studies indicate that people with haemoglobin genotype AS are not prone to malaria susceptibility when they have high level of immunity, but from this study high parasitaemia (+++) was observed from a pregnant woman with genotype AS. The findings in this study further support previous findings that dual infection with HIV and malaria may have serious consequences on the health of pregnant women. The dual infected pregnant women could be susceptible to many other infections. This is the evidence that where malaria and HIV occur together, infections interact. HIV worsens malaria infection by lowering the immunity of the infected pregnant women while malaria increases the risk of transmitting HIV from mother to child and also HIV viral load in the infected pregnant women is increased.

Desia et al. (2007) reported that in the areas endemic for malaria, at least 25% of pregnant women are infected with malaria, with the highest risk for infection and morbidity in primigravidas, adolescents, and those co-infected with HIV. Ned et al. (2005) also observed that the HIV co-infection has its impact on disease presentation, with an increased risk of complicated and severe malaria and death. Malarial infection in HIV-positive women is associated with higher levels of parasitemia, leading to a greater risk of severe anemia. Likewise, HIV viral load is increased, creating opportunity for infection and more severe disease (Briand et al., 2009). The moderate correlation that occurs between malaria and HIV implies that mothers infected with HIV are most likely to be infected with malaria due to the compromised immune system which makes them more susceptible to malaria infection.

RECOMMENDATION

It is therefore recommended that all pregnant women attending antenatal clinic in State Specialist Hospital Akure be tested for malaria parasite every month, in order to detect early, in case of any symptomatic or asymptomatic malaria infection, so as to be treated appropriately and prevent further morbidity and mortality associated with malaria. Intermittent preventive treatment should be provided for all pregnant women with preventive doses of an effective anti-malarial drug during routine antenatal clinic visits. Mosquito treated net should be made available free for the pregnant women. Training on how to use and maintain the net must be given as this will decrease the exposure to infective mosquito bites. More impressive public announcement and awareness should be made to the pregnant women in the public to attend good antenatal clinic for proper handling of their health and pregnancy from malaria and HIV.

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Conflict Interests

The authors declare that there is no conflict of interests

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