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ISID Small Grant Program Report

Plasmodium Falciparum DHFR Resistant Alleles and Opportunistic Parasitic Infection Among HIV Individuals on Cotrimoxazole Prophylaxis in Nigeria

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Introduction

Human immunodeficiency virus type 1 (HIV-1) infection and *Plasmodium falciparum* malaria are among the leading public health problems worldwide. Approximately 500 million people are infected with malaria and about 1.5–2.7 million deaths are recorded annually worldwide (Swan et al., 2005). Like malaria, the world's highest HIV infection rates are also found in Sub-Saharan Africa (SSA) where nearly 22.5 million (20.9–24.3 million) adults and children had HIV/AIDS in 2007 (UNAIDS, 2007). Also Gastrointestinal involvement in HIV/AIDS is almost universal, and significant disease occurs in 50–90% of patients while diarrhoea can sometimes be a presenting manifestation or a life threatening complication in HIV patients during the course of the disease (Gupta et al., 2008; Siddiqui et al., 2007).

The World Health Organization (WHO) and the United Nations program on HIV/AIDS recommended the use of cotrimoxazole (CTX) as prophylaxis for HIV-infected patients in Africa with symptomatic disease or CD4 cell counts < 500 cells/μL (UNAIDS/WHO, 2000). However, there has been concern that the widespread use of CTX for prophylaxis might accelerate the spread of resistance to Sulfadoxine Pyrimethamine (SP), which also is the currently recommended drug for the prevention of malaria in pregnancy (WHO, 2004) and also currently used in combination with artemisinin for the treatment of uncomplicated malaria in several African countries (Greenwood *et al*., 2005). It is assumed that the consequences of widespread CTX prophylaxis may promote corresponding population-level resistance resulting in the loss of the utility of CTX and other widely used antifolate class of drug including SP. This study was designed to determine the prevalence of *Plasmodium falciparum* parasite among HIV individuals and to characterize the dihydrofolate reductase (dhfr) gene that is thought to mediate malaria parasite resistance to antifolate class of drug. The study also determine the prevalence of opportunistic intestinal parasites and it's association to diarrhea among HIV individuals in Nigeria.

Current Study

Blood samples were collected from HIV individuals attending the HIV clinics in LAUTECH Osogbo and UCH Ibadan for malaria parasite detection. Finger pricked blood samples were collected directly unto a slide for microscope detection and about 2 drops were also spotted on filter paper (3 MM Whatman), labeled and kept in a dry clean container for molecular analysis. Positive samples were genotyped for dihdrofolate reductase (dhfr) gene mutations associated with SP resistance. The PCR assays for dhfr mutations include a primary and nested reaction to enhance specificity and sequencing to determine the mutations. For detection of intestinal parasites, stool samples were collected and processed using the direct saline and the formol-ether concentration technique. The Modified Ziehl-Neelsen technique was used for the identification of *Cryptosporidium* spp (Cheesbrough, 2000).

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For the malaria study a total of 3329 patients were screened for falciparum malaria parasite. Of these 2200 were females while 1129 were males. The overall prevalence of falciparum malaria in the study population was 9.7% (320/3329). Out of the 320 falciparum malaria parasites 146 were successfully genotyped for the points mutations (51, 59, 108 and 164) on dhfr gene associated with SP resistance. The prevalence of the mutant alleles for positions 51, 59 and 108 were 74%, 79% and 75% respectively. None of the parasite isolates had mutation at position 164. The overall prevalence of the triple dhfr mutation observed in the study was 73% (107/146) while only 4% had pure tripple wild alleles and 5% had mixed alleles.

For the detection of opportunistic intestinal parasites among the HIV individuals, a total of 96 confirmed HIV individuals from LAUTECH Osogbo were enrolled in this study. The mean age of the patients was 24.7 years and the sex distribution was 74 (77%) females and 22 (23%) males. The mean CD4 cell count of the patients was 352.6 cells/mm^3 of which 31.3% were severely immunosuppressed and had a CD4+ count of <200 Cells/mm^3. Of the total study population (n=96), 71(73.9%) harboured at least one parasite. The recovered parasites included *Cryptosporidium* spp. (54.2%), *A. lumbricoides* (9.4%), Hookworm (5.2%), *E. histolytica* (3.1%), *S. Stercoralis* (1%), and *Taenia* spp (1%). Double parasitic infections with *Cryptosporidium* were observed in a 13 (13.5%) patients. More males (68.2%) were infected than females (55.4%) but the difference was not statistically significant. There was a significant association (P=0.0001) between age group and intestinal parasite infection with older age groups having higher prevalence of infection. Patients with a *Cryptosporidium* (86.5%) infection presented a statistically strong association with diarrhoea (P =0.0001) while *A. lumbricoides* produced a weaker association (P = 0.0365). The highest prevalence of *Cryptosporidium* (90%) was observed among the patients that had the lowest CD4+ count of <200 Cells/mm^3. There was a significant association between *Cryptosporidium* spp. infection and CD4 count (P=0.0001).

The study observed low prevalence of malaria among patients that were taking daily cotrimoxaxole prophylaxis. Also there is widespread dhfr triple mutation among the HIV patients which did not correlate with cotrimoxaxole intake. *Cryptosporidium* infection was responsible for diarrhoea in 86% of HIV infected individuals in this study.

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