ISID Small Grants Program Final Report

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Identification of candidate single nucleotide polymorphisms (SNPs) in *Plasmodium falciparum* isolates showing false negative results using the ParaSight®-F dipstick.

Research Team

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Objectives

Evaluation of the sensitivity, specificity, efficiency and predictive value of ParaSight®-F dipsticks by comparison with standard microscopy and polymerase chain reaction.

Investigate whether the persistence of some HRPII antigens in circulating blood following parasite clearance is associated with persistent sub patent parasitemia and thus associated with drug resistance.

Identification of single nucleotide polymorphisms (SNPs) in coding and flanking regions of the HRPII gene in isolates of *Plasmodium falciparum*

Collation diagnostic outcome of patients' samples using the ParaSight®-F dipstick with presence or absence of SNPs in both coding and flanking regions of the HRPII genes of field isolates of *Plasmodium falciparum* in southwest Nigeria.

Background and Rationale of the Project

The recent development of rapid non-microscopic tests based on detection of *Plasmodium falciparum* histidine rich protein II (HRPII) antigens have been useful in diagnosis in many endemic countries. However, it has been reported that some isolates of *Plasmodium falciparum* do not express HRPII antigens (WHO, 2000) which may contribute to false negative results. The presence of Single nucleotide polymorphisms (SNPs) within coding and non-coding regions sequence of the parasite genome can result in a change in the structure and function of the protein encoded by the corresponding gene. In view of the potential implication of SNPs in plasmodium genome, efforts in the proposed research was devoted to ascertaining the existence of candidate SNPs in HRPII gene of *Plasmodium falciparum* isolates in Ibadan, Nigeria.

Patients aged 6 months to 12 years with clinical symptoms of acute uncomplicated malaria and microscopically confirmed *P. falciparum* infections were recruited into the study following informed consent from the parents/guardians. Enrolled patients were treated with dihydroartemisinin (4mg/kg for 5 days) and amodiaquine (25mg/kg body weight for 3 days) combination. Each patient was followed up for clinical and parasitological response to treatment on days 3, 7, 14, 21 and 28. Parasitological response was monitored by ParaSight-F dipstick and microscopic examination of Giemsa stained thick blood films.

Filter paper blood samples were collected for extraction and analysis of parasites' DNA prior to treatment and during the follow period. Microscopic assessment was compared with HRPII-based results. In case of discordance between these two diagnostic methods, PCR was performed for verification of the presence parasites in the blood sample. PCR amplification of HRPII genes in *P. falciparum* isolates with positive microscopy and negative HRP-II diagnostic result were performed using *P. falciparum HRPII* gene forward and reverse primers sequences. Sensitivity, specificity, negative and positive predictive values of microscopy and HRPII-based dipstick methods were determined.

Fifty patients were enrolled. Cure rate for dihydroartemisinin/amodiaquine was 94%. Using microscopy as the gold standard, the sensitivity and specificity of HRPII-based dipstick to diagnose malaria at enrollment were 94% and 100% respectively. The HRPII dipstick showed positive and negative predictive values of 100% and 94.4% respectively. The sensitivity of the dipstick reduced from 100% at enrollment to 32% and 0% on days 21 and 28 post-treatment respectively. The positive predictive value of the dipstick significantly reduced from 100% at enrollment to 0% on day 28 post-treatment.

Samples from five patients were positive by microscopy and negative with HRPII-based dipstick at enrollment. Parasitemia ranged from 468 to 2769 parasites/ microlitre blood in these patients. The five samples were positive by PCR (using the locus of msp2 gene). HRPII gene in

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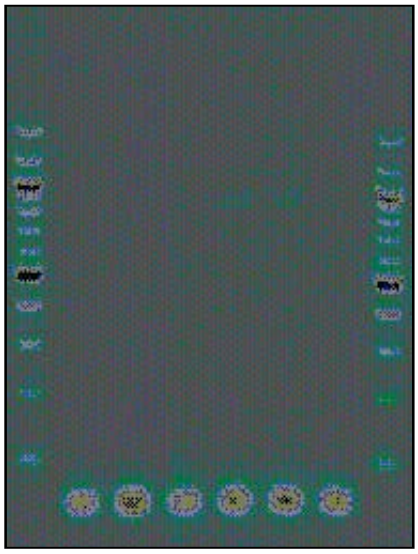

Fig.1. Gel electrophoresis of *P. falciparum* HRPII gene amplification. PCR amplification of the gene in field isolates and Controls DNA (*P. falciparum* strain K1 and 3D7) were seized against a 100bp DNA ladder (lanes 1 and 8). HRPII gene amplification in field isolates did not yield any product (lanes 2, 3, 4 and 7), while amplification of the controls DNA resulted in 900bp fragment (lanes 5 and 6).

these samples was not amplifiable by PCR for further sequencing and SNPs identification in flanking or coding regions. However the control DNA yielded positive results by PCR (Fig.1). It has previously been suggested that some field isolates of *P. falciparum* have the HRPII gene deleted (WHO 2000; New perspectives in malaria Diagnosis. WHO/CDS/RBM/2000.14). The lack of amplification of the HRPII gene of the 5 field isolates of *P. falciparum* for the identification of SNPs in the flanking and coding regions, is suggestive of deletion of this gene in the samples. However, further studies are needed to confirm this observation. The persistence of HRPII antigens in circulating blood despite the fast clearance of parasite by the combination of dihydroartemisinin-amodiaquine is also a serious concern for the use of this kit to monitor patients' responses to antimalarial drug treatment. ❖

For more information on the ISID Small Grants Program please see http://www.isid.org

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The Small Grants Program

is designed to fund pilot research projects by young investigators in developing countries. The goal is to support and foster the professional development of young individuals in the field of infectious diseases research by helping them to acquire additional skills and data to apply for other grants.