DNA-BASED STUDIES ON MALARIA

Thesis submitted in partial fulfillment of the requirements of the degree of Master of Philosophy of the University of Colombo, Sri Lanka.

by

CHARANI RANASINGHE

UNIVERSITY OF COLOMBO, SRI LANKA

1995

ABSTRACT

A simple malaria diagnostic technique based on non-radioactive DNA probes can greatly aid clinical and epidemiological studies in malaria. Utilizing sequence information on the small subunit ribosomal RNA of *Plasmodium falciparum* and *Plasmodium vivax*, short species specific oligonucleotides, complementary to rRNA genes were synthesized and covalently linked to biotin at the 5'terminus. Blood samples from patients infected with *P.falciparum* and *P.vivax* (0.06% - 0.5% parasitemia) were treated with acetic acid and the samples were dot-blotted onto nitrocellulose or nylon membrane, prior to the hybridization. The detection of the probes initially with streptavidin alkaline phosphatase and its substrate and secondly using a chemiluminecent detection system was not of any success. However, in a comparative study, the corresponding [^{32}P] labelled DNA probe also shown to lack adequate sensitivity.

A recently published, chemiluminescent detection system, using digoxigenin labelled pPF14 probe, targeting *P. falciparum* 21 base long repetitive DNA sequence of the telomeric region of the parasite chromosomes, was also tested after PCR

x#0

amplification. This method was sensitive enough for routine clinical and epidemiological studies but, lacked adequate specificity. Further research is therefore needed to develop suitable non-radioactive DNA based probes for malaria diagnosis.

The structural diversity in Sri Lanka of the major vaccine candidate molecules needs to be investigated before incorporating these antigens in a malaria vaccine for use in Sri Lanka. The genetic diversity of the 45 kDa glycosylated myristilated smaller surface antigen (GYMSSA) present on *P. falciparum* merozoites in natural isolates of Sri Lanka were investigated. A direct polymerase chain reaction amplification (PCR) of the GYMSSA gene from *P. falciparum* infected blood from patients was performed. After purification of the product a second round of PCR was performed with more internal primers incorporating M13 forward and the reverse primer sequences. Finally a simple cycle DNA sequencing was carried out using unlabelled M13 primers and α [^{35}S] - dATP by the dideoxynucleotide procedure. Two different DNA sequences were obtained substantiating the existence of at least the two major allelic families of GYMSSA in Sri Lanka.