RESEARCH NOTE

THE OCCURENCE OF DIHYDROFOLATE REDUCTASE (DHFR) POINT MUTATION (SER-108→ASN-108) IN MALAYSIAN ISOLATES OF *PLASMODIUM FALCIPARUM*

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Current *in vitro* methods for drug sensitivity testing of *Plasmodium falciparum* require viable parasites and this poses a problem for nation-wide drug sensitivity surveillance. The detection of molecular markers associated with drug resistance will provide a useful alternative due to the relative stability of deoxyribonucleic acids (DNA). Among the more widely used antimalarials that have lost their effectiveness are the 4-aminoquinolines, the sulfonamides and sulfones as well as the DHFR inhibitors such as pyrimethamine and proguanil (Cowman, 1995). Important mutations have been reported in various exons of the dihydrofolate reductase (DHFR) gene of *P. falciparum*. In two studies (Foote *et al*, 1990; Peterson *et al*, 1990), all pyrimethamine-resistant *P. falciparum* strains were found to have a point mutation at position 108 on the DHFR gene which could be detected by PCR. In this study, PCR using primers specific for the point mutation in DHFR at position 108 resulting in a change of amino acid from serine (Ser-108) to asparagine (Asn-108) was performed to identify the presence of this mutation in Malaysian isolates of *P.falciparum* and to determine whether there is an association between its presence and resistance of the parasite to Fansidar (SDX/PYR). The diagnostic primers were DIA-3 5'GAATGCTTTCCCAGC-3' (specific for Ser-108) and DIA-12 5' -GGAA-TGCTTTCCCAGT-3' (specific for Asn-108) which were used in conjunction with counterprimer SP1 5'-ATGATGGAACAAGTCTGCGAC-3' as reported by Peterson *et al* (1991). The PCR product for both primers is a 337 bp fragment.

Thirty whole blood specimens confirmed positive for *P. falciparum* by light microscopy (parasite count ranging from < 40-253,600 asexual stages/0-520 sexual stages per µl blood) from patients in Tawau Hospital, Sabah, East Malaysia were collected for the study. Six other samples were collected from patients with severe and complicated malaria before treatment with Fansidar. A second blood film was collected from each of these patients one week after treatment with Fansidar to monitor the parasitemia. In addition, 9 other *P. falciparum* isolates were from patients in Gombak Hospital and Hospital Kuala Lumpur which were cultured *in vitro* and their sensitivity to SDX/PYR determined by the WHO *in vitro* mictotest. Blood samples from healthy donors were used as controls. The blood samples (300 µl) were processed according to the method of Peterson *et al* (1991) for use in the PCR. Forty-five cycles of amplification were performed, each consisting of denaturation at 94°C for 30 seconds, renaturation at 56°C for 45 seconds and extension at 74°C for 45 seconds. Amplified products were stained with ethidium bromide and photographed after electrophoresis in 1% agarose gel.

The results of the PCR for the 30 *P. falciparum* isolates from Tawau Hospital are as summarized in Table 1. It was observed that 27 out of the 30 samples had the Asn-108 mutation and of these 19 also had the wild type marker (Ser-108). Table 2 shows the PCR results obtained with the other 6 isolates from patients who received Fansidar treatment. Isolates from 5 of these patients appeared to be sensitive to Fansidar (not RI) as no parasites were seen in their blood film a week after treatment; although one however, had a low level of gametocytes. Of these, 3 (F1-F3) had both the wild type and mutant markers while 1 (F4) was positive for Asn-108 while F5 had only the Ser-108 marker. Patient no. F6 who continued to have a parasitemia one week after Fansidar treatment was positive for Asn-108. For the *in vitro* cultured isolates from West Malaysia (Table 3), the drug sensitivity levels

Table 1

Occurrence of Ser-108 and Asn-108 in *Plasmodium falciparum* isolates from Tawau Hospital, Sabah.

Patient reference	Parasite count per μl blood (no. asexual/no. sexual)	PCR Results	
		Ser-108	Asn-108
TH1	253,600/400	+	+
TH2	< 40/0	+	+
TH3	7,840/40	-	+
TH4	480/0	+	+
TH5	88,480/520	+	+
TH6	39,680/320	-	+
TH7	5,320/40	+	+
TH8	80/0	+	+
TH9	120/40	+	+
TH10	4,480/400	+	+
TH11	39,360/0	+	+
TH12	7,600/0	+	+
TH13	5,760/0	+	+
TH14	80/0	+	-
TH15	< 40/0	-	+
TH16	8,800/40	+	+
TH17	39,520/80	+	+
TH18	6,720/560	+	+
TH19	4,040/280	+	+
TH20	4,480/0	+	+
TH21	46,080/80	-	+
TH22	360/0	+	-
TH23	6,760/280	-	+
TH24	132,320/160	-	+
TH25	27,760/40	-	+
TH26	71,200/0	-	+
TH27	36,800/240	-	+
TH28	152,000/0	-	+
TH29	7,200/40	-	+
TH30	3,440/40	+	-

ranged from 100-10,000 pmol/well SYX/PYR. All the isolates had the Asn-108 mutation but again, there was some heterogeneity of markers with 6 having also the wild type (Ser-108).

From the results obtained, the Asn-108 mutation appears to be quite prevalent in Malaysian isolates of *P. falciparum* from both East and West Malaysia. Lokman Hakim *et al* (1996) reported that *P. falciparum* isolates from various endemic areas in West Malaysia showed a fairly high resistance rate of 47.4% to SDX/PYR with the proportion of the severe type of resistance (RII and RIII) being 37%. The level of resistance was however, not significantly related with pre-treatment parasite densities. From the PCR results of the 6 patients treated with Fansidar, there appeared to be no clear association between the presence of the Asn-108 mutation and Fansidar resistance. All the 9 *in vitro* cultured isolates from West Malaysia had the Asn-180 mutation although their sensitivity to SDX/PYR ranged from 100-10,000 pmol/well. A large proportion of the isolates studied had both Ser-108 and Asn-108 indicating mixed populations of pyrimethamine-sensitive and resistant parasites. Peterson *et al* (1991) also reported the occurrence of both markers in a small number of *P. falciparum* isolates from

Table 2

Detection of Ser-108 and Asn-108 in *P. falciparum* isolates from patients in Tawau Hospital before Fansidar treatment.

Patient reference	Parasite count per µl blood		PCR results on blood sample before treatment	
	Before Fansidar treatment	1 week after treatment	Ser-108	Asn-108
F1	5,320/40	Negative	+	+
F2	8,800/40	Negative	+	+
F3	4,040/400	0/40	+	+
F4	7,840/40	Negative	-	+
F5	33,440/40	Negative	+	-
F6	< 40/0	40/40	-	+

Table 3

Occurrence of Ser-108 and Asn-108 in *in vitro* cultured *P. falciparum* isolates from malaria patients from West Malaysia.

Patient reference	Highest concentration of Fansidar (pmol/well) with schizont growth	PCR results	
		Ser-108	Asn-108
ST187	100	+	+
ST284	200	-	+
ST227	200	+	+
ST228	1,000	+	+
ST458	3,000	+	+
ST112	3,000	+	+
ST280	10,000	-	+
ST313	10,000	+	+
ST312	10,000	+	+

the Brazilian Amazon. From this study, it appears that the Asn-180 mutation should not be used alone as a marker for pyrimethamine resistance in Malaysian isolates of *P. falciparum*. Other mutations have been reported in the DHFR gene that are related to pyrimethamine resistance (Basco *et al*, 1995; Thaithong *et al*, 1992). Although there is some indication that Asn-108 may confer some degree of resistance to pyrimethamine in these isolates, additional point mutations in the DHFR gene for example, Asn-51 to Ile-51 and Cys-59 to Arg-59 (Peterson *et al*, 1988) need to be examined to determine whether a cumulative effect of mutations is responsible in conferring high antifolate resistance in Malaysian *P. falciparum* isolates.

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