

Original article

Characterization of the Population Demographics and the MSP-1 Block 2 Allele Gene Frequencies of *P. falciparum* Infected Individuals in Davao, Philippines

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Abstract: *Plasmodium falciparum* is one of the causative agents of malaria in humans. This parasite causes the most severe forms of the disease. In order to combat the disease, it is important to have knowledge about the parasite and its interaction with its host. In this study, we profiled 74 patients admitted to hospital in Tagum, Davao, Philippines who were confirmed to be infected with *P. falciparum*. We correlated the age, sex and parasite load with malaria severity and show that among these, only sex is correlated with disease severity in this population. In addition, we profiled the MSP-1 block 2 allele distribution in the population and found that the most abundant allele form was K1, followed by MAD20. The RO33 allele form was the rarest allele in this population.

Key words: *Plasmodium falciparum*, MSP-1, severe malaria, endemic area

INTRODUCTION

Malaria is an ancient disease that continues to plague the human species to this day. The most severe form of malaria in humans is caused by *Plasmodium falciparum*. Approximately 2.4 billion people worldwide are at risk of being infected with *P. falciparum*. It is also estimated that the parasite causes illness in 500 million individuals per annum and approximately 1.2 million deaths per year. Children below five years of age and pregnant women make up the majority of individuals that succumb to this disease [1, 2].

To effectively combat malaria, much has to be learned about the parasite that causes it and its interaction with the human host. Although there have been many studies describing the profile of patients and parasite genetics in endemic regions in Africa, South America and some countries in Asia [3–9], there is little information about the population demographics and parasite genetics of malaria endemic regions in the Philippines. In this study, we investigated the

demographics of a population of malaria patients from Tagum, Davao, a malaria endemic region in the Philippines, and determined the frequency of the *P. falciparum* MSP-1 block 2 allele forms present in this population. In addition, we investigated the correlation between age, sex, parasite load, and allele form and malaria severity.

MATERIALS AND METHODS

Population

A total of 74 malaria patients at the Davao Regional Hospital in Davao, Philippines consented to be part of the study. *P. falciparum* infection in each of these patients was confirmed via microscopic identification of the parasite using Giemsa-stained blood smears. Of these patients, 26 manifested at least one of the following symptoms of severe malaria (i.e., change in behavior, hypoglycemia, seizures, impaired visceral function, acidosis, difficulty breathing, and severe anemia) and were classified as severe malaria

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cases while the remaining 48 were classified as uncomplicated or mild malaria cases. The age, sex, and clinical findings of each patient were also recorded.

DNA extraction and PCR amplification

P. falciparum genomic DNA was extracted from peripheral blood taken from each patient using the one-step PCR kit from Takara (Japan) according to the manufacturer's instructions. The extracted DNA was then mixed with 50 μ l of the 2x PCR solution (Takara, Japan) along with 48 μ l of water and 33 nanomoles each of the MSP-1 specific primers as described by Robert, *et al.* [5]. The tube was then placed in the thermocycler (PTC-100, USA) and subjected to an initial melting of 94°C for 20 minutes. This was followed by 31 cycles of repeated denaturation at 94°C for 30 seconds, annealing at 55°C for one minute and extension at 72°C for one minute. The PCR products were then visualized on a 2% agarose gel stained with ethidium bromide.

MSP-1 block 2 allele typing

Samples positive for the MSP-1 gene were subjected to a second PCR reaction using primers specific for the MAD 20 allele family, the K1 allele family or the RO33 allele family as described by Robert, *et al.* The PCR products were visualized using a 2% agarose gel, and the band sizes of the PCR products were calculated using linear regression.

Statistical Analysis

Chi-square (χ^2) tests were performed to determine the correlation between allele frequencies and malaria severity and between age and malaria severity. On the other hand, the Fischer's exact test was done to correlate malaria severity with gender, and parasite load. Both tests were performed using GraphPad InStat v. 3.10. A p-value of < 0.05 was considered significant.

RESULTS

Patients

Of the 74 patients who participated in the study, 26 (35%) suffered from severe malaria while 48 (65%) suffered from a milder form of the disease. Patients with the severe form of the disease exhibited the following signs and symptoms: splenomegaly (42% of patients with severe malaria), hepatomegaly (35%), dark colored urine (31%), respiratory distress (31%), convulsions or seizures (19%), change in behaviour (19%), and loss of consciousness (3%). The mean parasite was 60,733 ring forms per ml and 148,903 for patients with mild malaria and severe malaria,

respectively. Sixty (80% of samples) individuals had parasite counts below 100,000 and only 14 (19%) had counts above 100,000. Figure 1 shows the distribution of mild and severe cases between these two groups. The statistical analysis revealed no significant correlation between parasite count and disease severity.

Sex and malaria severity

Forty-five (61%) of the samples collected were from males while only 29 (39%) of the samples were from females. Figure 2 shows the distribution of severe and mild cases between the two sexes. Statistical analysis using the Fisher's exact test revealed a significant correlation between sex and disease severity in the population tested, with men being more likely to contract a severe form of the disease than women.

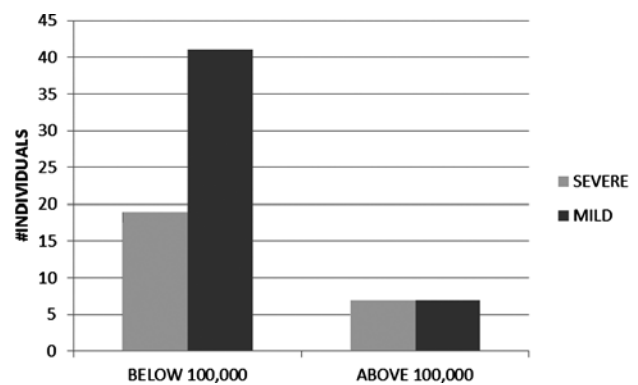


Fig. 1. Parasite load and malaria severity. The distribution of mild and severe malaria cases based on parasite load (ring form). Statistical analysis reveals that there is no significant correlation between malaria severity and parasite load in this population.

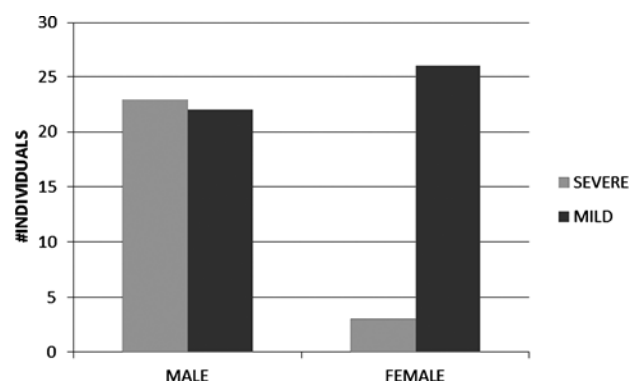


Fig. 2. Sex and Malaria Severity. The distribution of mild and severe malaria cases between male and female patients. Statistical analysis shows that sex and malaria severity are correlated ($p < 0.05$) with males more likely to develop severe malaria than females in this population.

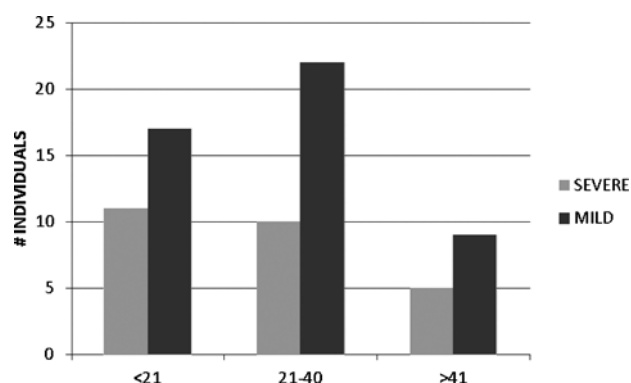


Fig. 3. Age and malaria severity. The distribution of mild and severe malaria cases based on patient age. Statistical analysis reveals that there is no significant correlation between patient age and malaria severity in this population.

Age and malaria severity

The samples were classified into three age groups: <21, 21–40, and >40. Figure 3 shows the distribution of the patients among these age groups. The largest group of patients was the 21–40 age group (43%) followed by the <21 (28%) and finally, the >40 group (19%). Statistical analysis using the chi-square test revealed no significant correlation between age and malaria severity.

Allele typing of the MSP-1 gene

The MSP-1 gene was typed using Block 2 allele-specific primers. All of the major allele types (K1, MAD20, and RO33) were detected in the population. Table 1 summarizes the results of the allele typing. The alleles were then classified according to the size-based classification of Robert, *et al.* [5].

A total of 35 samples (47%) were positive for the K1 allele, with 18 (51%) of those positive for K1 coming from patients with mild malaria and 17 (49%) from those with severe malaria. All of the previously reported subtypes of the K1 allele were found in the population studied, except for allele types g and j.

Twenty-seven (36%) of the samples were positive for the MAD20 allele, including 15 (55.5%) from patients with severe malaria and 12 (44.5%) from those with mild malaria. All previously reported allele types for MAD20 were observed in the population, along with two allele types not reported by Robert, *et al.* [5] characterized by 117 bp and 137 bp PCR fragments respectively.

Only nine (11%) of samples tested positive for the RO33 allele with eight (89%) coming from patients with severe malaria and only one (11%) from those with mild ma-

Table 1. Distribution of alleles and allele subtypes

Allele	Subtype	Severe cases	Mild cases
K1		17	18
	a	2	6
	b	5	5
	c	3	3
	d	5	1
	e	2	2
	f	2	2
	g	0	0
	h	2	0
	i	1	1
	j	0	0
MAD20		15	12
	117 bp	1	0
	137 bp	1	2
	a	5	5
	b	5	10
	c	0	1
	d	6	5
RO33		8	1
	109 bp	1	0
	a	3	1
	b	5	0
	c	3	0
untyped		2	24

laria. All of the previously reported allele types were observed in the population, along with an allele characterized by a 109 bp PCR product which was not observed by Robert, *et al.* [5].

Statistical analysis of the data revealed no correlation between allele type and malaria severity or between allele type and parasitemia.

Unfortunately, we were unable to type 26 (35%) of the samples, two from the patients with severe malaria and 24 from the patients with mild malaria. Ten (10) of the samples could not be typed, possibly due to the degradation of the DNA during the transport and storage of the whole blood samples. However, we were able to recover DNA from 16 of the samples, as evidenced by the positive result of the amplification reaction with MSP-1 specific primers prior to allele typing, but these samples yielded no PCR products for the allele typing reactions.

DISCUSSION

In this study we describe the profile of a population of patients from a malaria endemic area (Table 2). Our findings reveal a correlation between sex and malaria severity in this population, with males being at a higher risk of developing severe malaria than females. On the other hand, no correlation was observed between age and malaria severity or between parasite load and malaria severity.

We also determined the frequency of the three allele families of block 2 of the MSP-1 gene. The MSP-1 gene codes for the predominant protein found on the surface of

the blood stage of the parasite believed to play a role in RBC invasion by the parasite. Previous studies have shown that the RO33 allele of block 2 is associated with high levels of TNF- α in patients, which in turn is correlated with the development of severe malaria [5].

The predominant MSP-1 block 2 allele family present in the patients from the Davao Regional Hospital in Tagum was the K1 allele, followed by the MAD20 allele. The RO33 form of the MSP-1 block 2 gene was very scarce in the population, with only 13.24% of the samples testing positive for this allele family. These results differ widely from those of the population of hospital patients studied in

Table 2. Summary of population demographics.

	SEVERE		MILD	
	# individuals	%	# individuals	%
SPLENOMEGALY	10	38.5	0	0
HEPATOMEGALY	10	38.5	0	0
DIFFICULTY BREATHING	8	30.8	0	0
SEIZURES	6	23.1	0	0
DARK COLORED URINE	5	19.2	0	0
CHANGE IN BEHAVIOR	4	15.4	0	0
PALLOR	4	15.4	0	0
CEREBRAL MALARIA	2	7.7	0	0
ANEMIA	2	7.7	0	0
THROMBOCYTOPENIA	2	7.7	0	0
JAUNDICE	1	3.8	0	0
COMA	1	3.8	0	0
HYPOGLYCEMIA	0	0.0	0	0
PARASITEMIA				
<100,000	19	73.1	41	85.4
\geq 100,000	7	26.9	7	14.6
AGE GROUP				
<21	11	42.3	17	35.4
21–40	10	38.5	22	45.8
>40	5	19.2	9	18.8
SEX				
MALE	23	88.5	22	45.8
FEMALE	3	11.5	26	54.2
MSP-1 BLOCK 2 ALLELE				
K	5	19.2	11	22.9
M	4	15.4	6	12.5
R	2	7.7	0	0.0
KM	7	26.9	6	12.5
KR	2	7.7	1	2.1
MR	1	3.8	0	0.0
KMR	3	11.5	0	0.0
UNTYPED	2	7.7	24	50.0

Table 3. Distribution of MSP-1 block 2 allele families in selected populations

LOCATION	TYPE OF POPULATION	# OF ISOLATES	%K1	%MAD20	%RO33
Tagum, Philippines (this study)	Hospital patients	74	47	36	12
Southern Vietnam [9]	Ethnic communities (moderate endemicity)	238	10	26	6
Thailand [7]	Hospital patients	368	14	34	2
Honduras [6]	Ethnic community	56	46	73	0
Brazilian Amazon [8]	Ethnic community	181	33	22	45
Northern Tanzania [4]	Ethnic communities (high endemicity)	79	56	38	33
Senegal [5]	Hospital patients	86	37	20	59

Dakar, Senegal where 37.21%, 19.77%, and 59.30% of the 86 samples were positive for the K1, MAD20 and RO33 allele families, respectively [5]. These results are also quite different from those of a study done in an ethnic community in Colon, Honduras [6] where 46.43% and 73.21% of the 56 samples studied were positive for the MAD20 and K1 allele families, respectively, and not a single RO33-like allele was detected. Even the study performed among hospital patients in Thailand by Snounou, *et al.* [7] shows a slightly different result, with the majority of alleles coming from samples representing the MAD20 allele family and the least number of alleles coming from the RO33 family. A comparison of the studies in different populations is presented in Table 3.

The differences in the profile of the MSP-1 block 2 allele of the *P. falciparum* parasite present in different populations around the world may be due to several factors, including variations in the parasite genome which may be specific to each endemic area and alter the population dynamics of the parasite and differences in host-factors between one population and those in the rest of the world, e.g., HLA types. Future studies are underway to identify novel genetic markers specific for the isolates gathered from malaria endemic areas in the Philippines to supplement the results of this study.

It is also important to note that we were unable to type a large number of samples. We attributed this to the degradation of the DNA due to non-ideal transport and storage conditions of the blood sample during collection and transport or the possible presence of recombinant forms or mutant forms of the block 2 allele. However, attempts to type the samples using combinations of the allele-specific primers have been unsuccessful. We hope that the aforementioned studies designed to identify Philippine-specific genetic markers may also yield some information on the possible identities of the MSP-1 block 2 allele of the untyped samples.

The low incidence of the RO33 allele, however, seems to reflect the low incidence of mortality (0.4 per 1,000 peo-

ple) and morbidity in the country [2]. On the other hand, the population of the Senegal study that has a higher incidence of the RO33 allele also has a higher incidence of severe malaria [5]. We are currently investigating the possible molecular mechanisms that may be the underlying cause of this apparent association between the frequency of the RO33 allele and the incidence of severe malaria.

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