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Full Length Research Paper

Changes in Plasmodium Falciparum Population Dynamics in Two Populations at Different Time Periods in Ibadan, South-west Nigeria

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ABSTRACT

Changing the malaria epidemiology will affect the genetic diversity of *Plasmodium falciparum.* We studied the association between diversity at the merozoite surface protein 2 loci and the severity of disease in childhood malaria in two populations and at different time periods in Ibadan, southwest Nigeria. Population A comprised of 164 children (75 acute uncomplicated malaria (UM), 48 cerebral malaria (CM) and 41 severe malarial anaemia (SMA), while Population B comprised of 225 children (115 UM, 55 CM, 55 SMA). Results showed a high level of genetic diversity and multiplicity of *P.falciparum* infections in the two populations. Polyinfections were common in the 2 populations but different (93% for PA and 52% for PB), the mean multiplicity of infections was different (3.98 per infected person for Population A and 1.80 for Population B. The presence of polyinfections was significantly lower only in the SMA group in Population A, p=0.007 but significantly lower in the CM, p=0.003 and SMA groups, p=0.000 in Population B. The presence of FC27 and 3D7 alleles was a significant predictor of SMA in Population A but not in Population B. The absence of polyinfections (single infections) was found to be a strong common factor or predictor of severe malaria in the two populations. We conclude that presence of single infections are associated with the development of severe malaria. In addition, malaria control activities have a great impact on the changing parasite population dynamics.

Key words: msp2, Plasmodium falciparum, severe malaria, genetic diversity

INTRODUCTION

The most common clinical presentations of severe malaria in sub-Saharan Africa are severe malarial anaemia, cerebral malaria and respiratory distress. These clinical presentations co-exist but the clinical spectrum varies depending on the intensity of transmission (Snow and Marsh, 2002). The most at-risk group are children under the age of five and these vary across different transmission intensities (Carneiro *et al*., 2010, Roca-Feltrer *et al*., 2010). What determines these clinical presentations, why some individuals develop the severe disease even with scanty parasitaemia and others have only the mild form even with heavy parasitaemia is not fully known. This has however been associated with the parasite and host factors (Mackinnon *et al*., 2005).

P. falciparum shows considerable diversity at several genetic loci, including MSP-1, MSP-2 genes (Felger *et al*., 1999, Mwingira *et al*., 2011, Kiwuwa *et al*., 2013). This characteristic antigenic variation and the complexity of the parasite life cycle had hindered much the development of an effective vaccine. There is an increased interest in the study of genetic variation and evolution of the malaria parasites in recent years with the view of developing a good candidate based vaccine. The most polymorphic genetic loci is *msp-2* gene and it has been suggested that the *msp2* gene

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could alone, serve as a marker for *P.falciparum* diversity (Felger *et al*., 1999, Felger *et al*., 2003). Genetic diversity of *P.falciparum* populations and the complexity of infection have been shown to be age-dependent and vary according to the intensity of transmission and outcome of infections in different geographical regions (Engelbrecht *et al*., 1995, Issifou *et al*., 2003, Amodu *et al*., 2005, Amodu *et al*., 2008, Kiwanuka *et al*., 2009, Hamid *et al*., 2013).

Previous studies have documented the use of genotyping the highly polymorphic Plasmodium falciparum msp1 and msp2 genes to describe variability in alleles within a population of parasites (Carlsson *et al*., 2011, Ibara-Okabande *et al*., 2012) In addition, genotyping has been used for drug resistance surveillance and to classify treatment outcome (Al-abd *et al*., 2013, Gosi *et al*., 2013). In Nigeria, in spite of widespread use of Artemisin-based combined therapies (ACTs), little is known of the presence or emergence of artemisinin resistance. With the increased rate of resistance in many countries, it is therefore important for drug surveillance studies using genotyping to be carried out in Nigeria. To contribute to this growing body of knowledge, we present here base-line studies of the genetic diversity of *P.falciparum* using the most polymorphic parasite genetic marker msp-2. We present results from two different populations at different times; a short while (1998-2000) before the antimalarial policy change from Chloroquine to ACTs and within a short period after the change (2003-2005) and the association with clinical severity of malaria.

METHODOLOGY

We studied the association between diversity at the merozoite surface protein 2 loci and the severity of disease in childhood malaria in two populations and at different time periods in Ibadan, a city in southwest of Nigeria, holo-endemic for malaria. An informed consent was obtained from the parents or guardian of the patients prior to recruitment. Ethical approval was obtained from the joint UI/UCH Ethical Committee. Demographic information and clinical history information obtained from caregivers of children and clinical examinations done were recorded in a well-structured Case record form.

Study Populations: Population A: 164 children (75 acute uncomplicated malaria (UM), 48 cerebral malaria (CM) and 41 severe malarial anaemia (SMA), were enrolled between the rainy seasons (May-September) of 1998-2000. Population B a subset of a previously published study (Amodu *et al*., 2008) comprised of 225 children (115 UM, 55 CM, 55 SMA) were recruited between the rainy seasons (May-September) of 2003-2005.

Blood Collection: About 500 ml of venous blood were collected from each child, into a sterile EDTA tube, for parasitological and haematological investigations. Thick blood smears stained with Giemsa were prepared for each child and examined for trophozoites of *P.falciparum.* Parasite densities were calculated based on assumed total WBC of 8000/µL. For analysis of the P. falciparum DNA in each sample, dried blood spots samples were obtained from children presenting with microscopically confirmed *P. falciparum.* The filter blood spots were air dried and stored at -20^{0}C. *P.falciparum* infections were confirmed with a species-specific Polymerase Chain Reaction (PCR).

DNA Extraction and Gene Amplifications: Total DNA was extracted from the dried blood spots with methanol and genotyping of the genes *msp2* was by nested PCR. The amplicons were separated by electrophoresis in 1.2% agarose gel, stained with ethidium bromide and visualized by trans-illumination with ultraviolet light. Amplicon sizes were then estimated, by comparing the positions of the sample bands with those of the bands in a 100-bp DNA ladder run on the same gel. The expected amplicon size range was 260–540 bp for the FC27 allelic family and 170–470 bp for the 3D7.

Statistical Analysis

All the data analyses were conducted using SPSS version 16.0. Descriptive statistics (means, S.E., medians and ranges) were computed for continuous variables and frequencies were computed for the categorical variables. Group comparisons of the continuous and categorical variables were done Chi-square tests. Multinomial logistic regressions were carried out with disease severity as the outcome and 'uncomplicated malaria' as the reference category.

RESULTS

General characteristics of the population

Population A comprised of 90 (55%) males and 74 (45%) females. Of these 75 (45%) presented with acute uncomplicated malaria, 48 (29%) presented with cerebral malaria and 41 (25%) with severe malaria anaemia based on the criteria of the World Health Organization (World Health Organization 2000). The median age for the total population was 26.0 months. The median age for the UM and SMA group was 24.0 months and 37.0 months for the CM group. The geometric mean parasite density for the UM group

7079/µl, CM group 5623/µl and 3467/µ for the SMA group l p<0.021. Population B comprised 225 children of whom 113 (50.2%) were males and 112 (49.8%) were females with a median age of 32 months. Based on the criteria of the World Health Organization, 115 were classified as acute uncomplicated malaria (UM), 110 as severe malaria (SM) - 55 cerebral malaria (CM), 55 severe malarial anaemia (SMA). The three categories of subjects differed significantly in age parasite density and hematocrit. The geometric mean parasite density for the UM group was 6,739/µl and 30,711/µl for the SM group.

Multiplicity of infections and presence of FC27 and 3D7

Table 1 shows that the complexity of infection, frequency of poly-infection, and presence of FC27 and 3D7 alleles by clinical category in the two populations.

In Population A, a total of 684 infections were detected in 164 isolates ranging from 0-10 infections per person. The mean multiplicity of infections was 3.98 per infected person. In the population, eight individuals had no msp-2 alleles, 4 individuals had one infection, while some individuals in the population had 4-5 infections (21% of the total population had 4 infections and 24% had 5 infections). The majority of the population had more than one distinguishable infection (93%) and one subject had 10 different msp2 alleles. The mean multiplicity of infections among the uncomplicated malaria group was 3.74 versus 4.24 among the severe malaria group. In Population A, poly-infections (poly-clonality) were significantly lower in the SMA group when compared with the other two groups, with the UM and CM groups being comparable. The SMA group was defined by highest frequency of FC27 alleles (found in nearly all isolates in the group) and the lowest frequency of 3D7 alleles when compared with isolates from the other two groups.

In Population B, the mean multiplicity of infections was 1.80 per infected person. About 52% of the population had more than one distinguishable infection, polyinfections of different genotypes. About one-half of the subjects had one infection (28% had 2 infections and 12% had 3 infections) and one subject had 6 different infections. One hundred and twenty one of the study samples (53.8%) were positive for the FC27 alleles, 155 (68.9%) for the 3D7 alleles. In Population B, poly-infections (poly-clonality) were significantly the lowest in the CM group followed by the SMA group. The UM group was defined by highest frequency of 3D7 alleles (found in nearly all isolates in the group) and the highest number of polyinfections.

Association of polyinfections and msp2 alleles with severity of malaria

Table 2 shows a multivariate logistic regression of the severity of malaria on the complexity of malaria infection and the presence of FC27 and 3D7 alleles using the UM group as a reference category for both populations.

In Population A, multiplicity of infection and polyinfections when adjusted for age, sex and parasitaemia, were negatively correlated with the SMA group that is, a lower number of polyinfections and complexity was strongly associated with the clinical outcome of severe malaria anaemia (p = 0.007). The presence of the 3D7 alleles was also negatively associated with the SMA group (p = 0.030)

Table 1:
Association of Complexity of Infection, Polyinfection and The Presence of Alleles With Severity Of Malaria

Characteristics	Severity of malaria			p
	UM	CM	SMA	
Population A				
Multiplicity of infections	2.8	3.4	1.9	<0.001
Polyinfection [no (%)]	63	40	23	0.001
Presence of FC27 alleles [(%)]	35	11	40	<0.001
Presence of 3D7 alleles [(%)]	45	28	13	0.009
Population B				
Multiplicity of infections	2.1	1.3	1.5	<0.001
Polyinfection [no (%)]	63	25	40	<0.001
Presence of FC27 alleles [(%)]	50	52	57	0.659
Presence of 3D7 alleles [(%)]	78	56	58	<0.001

The three groups also differed significantly in complexity of infection and the presence of FC27 and 3D7 alleles.

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Table 2:
Multinomial Logistic Regression of Malaria Severity on Presence of Fc27 and 3d7 Alleles

	Regression coefficient	SE	p
Population A			
Cerebral malaria			
Polyinfections	0.278	0.134	0.109
FC27 alleles	-0.630	0.463	0.173
3D7 alleles	0.257	0.459	0.576
Severe malarial anaemia			
Polyinfections	-0.676	0.250	<0.007*
FC27 alleles	3.838	1.071	<0.001*
3D7 alleles	-1.173	0.526	0.030*
Population B			
Cerebral malaria			
Polyinfections	-2.129	0.471	<0.001*
FC27 alleles	0.734	0.493	0.136
3D7 alleles	-0.169	0.494	0.732
Severe malarial anaemia			
Polyinfections	-1.261	0.425	0.003*
FC27 alleles	0.709	0.438	0.106
3D7 alleles	-0.426	0.443	0.337

*All models included adjustment for age and parasite density. Reference category: uncomplicated malaria; controlled for age and parasite density; * P < 0.05. Reference category: Uncomplicated malaria; *p < 0.05; +Adjusted for age, sex and parasite density. Reference category for alleles = presence of the alleles*

The presence of the FC27 alleles were however strongly correlated with the SMA group (correlation coefficient = 3.838), showing a strong association with the SMA group ($p < 0.001$). The polyinfections and presence of FC27 alleles after adjustment for age, sex and parasite density were not significant predictors of cerebral malaria. However, for severe malarial anaemia, the polyinfections, presence of FC27 alleles and presence of 3D7 alleles were all significant predictors. After adjustment for age, sex and parasite density, they remained independent significant predictors suggesting that their association is independent of these potential confounders.

Results for Population B show that polyinfections were significantly lower in the CM and SMA groups when compared with UM. The UM group was defined by highest frequency of 3D7 alleles when compared with isolates from the other two groups (p=0.000). The association of the 3D7 and FC27 alleles with the clinical groups was not significant. The presence of polyinfections was negatively associated with CM and SMA. The absence of poly-infections was strongly associated with the development of severe malaria, when compared with uncomplicated malaria as a reference category, 4 times increased risk in severe malaria anaemia and 9 times increased risk for cerebral malaria. Thus, absence of polyinfections /presence of only single infections was found to be an independent predictor of severe malaria.

DISCUSSION

Plasmodium parasites undergo clonal antigenic variation, resulting in multiclonal infections (Farnert *et al*., 2001, Elbasit *et al*., 2007, Babiker and Walliker, 1997). Genetic diversity between different parasite clones has been demonstrated in many field studies and appears to vary geographically (Hamid *et al*., 2013, Kiwanuka *et al*., 2009, Elbasit *et al*., 2007). We present data comparing the genetic diversity of *msp2* in two different populations in Ibadan at different time periods, population A from a period before and population B from a period shortly after the national antimalarial drug policy change in 2004 from choloroquine to the artemisinin combination therapy (ACTs). Findings from this study showed a high level of genetic diversity and multiplicity of *P.falciparum* infections in the two populations. Polyinfections were common in the 2 populations but different (93% for PA and 52% for PB), though the mean multiplicity of infections was different (3.98 per infected person for Population A and 1.80 for Population B. Allelic amplification of the *msp-2* gene is one of the important means of characterising parasite population structure (Felger *et al*., 1999, Smith *et al*., 1999). This can aid monitoring the spread and genetic background of drug resistance, the changing epidemiology and the conditions of transmission (Al-abd *et al*., 2013, Ibara-Okabande *et al*., 2012, Mwingira *et al*., 2011). In our

study, the children were recruited at different times (Population A in 1998-2000 and Population B in 2003-2005) from the same area. Control programmes were just being implemented and intensified with increased activities culminating in the national antimalarial drug policy change (in 2004) within the time periods. Knowledge is assumed to have increased as a result of these activities and enlightenment campaigns. Therefore these activities, however little, may have resulted in a changing parasite population dynamics. Consequently any malaria control measure would have an impact on these different parasitological parameters.

The presence of polyinfections was significantly lower only in the SMA group in Population A but significantly lower in the CM and SMA groups in Population B. The presence of FC27 and 3D7 alleles was a significant predictor of SMA in Population A but not in Population B. Findings from this present study showed the absence/lack of polyinfections were however significantly associated with the development of severe malaria, both in cerebral malaria and severe malaria anaemia. The absence of polyinfections was found to be a strong common factor or predictor of severe malaria in the two populations. This is similar to findings from a subset of the data from previous studies done in our setting (Amodu *et al*., 2008).

We therefore conclude that polyinfections are negatively associated with the development of severe malaria. In addition, malaria control activities play an immense role in impacting changing parasite population dynamics. With these findings, it is therefore necessary to carry out these studies with data to present a more recent picture of the malaria parasite population dynamics and drug resistant patterns if any, and if widely spread in Nigeria with regards to the continuous use of ACTs.

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