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RESEARCH ARTICLE

PROTECTION AFFORDED BY SICKLE-CELL TRAIT AGAINST ASYMPTOMATIC MALARIAL INFECTION IN BURKINA FASO

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ABSTRACT

Background: Malaria is a major public health problem in Burkina Faso because every yearthe *burkinabè* population continue to bear a heavy burden. Malaria infection depends on the parasite's ability to interact with the host's red blood cells for its development. In population living in malaria endemic areas, sickle cell trait (HbAS, HbAC and HbCC) is considered to be a factor in malaria resistance. The present study aims to assess the involvement of sickle cell hemoglobin S and C in the protection against asymptomatic *Plasmodium falciparum* infections in Burkina Faso. **Methodology**: The study population consisted of 182 participants. A volume of 4 ml of venous blood was collected from each participant and referred to CERBA for the determination of parasitaemia, hemogram, blood group/rhesus and hemoglobin electrophoresis. **Results:** The age group of the study population was distributed as follows: less than 5 years (45.6%), 5 to 15 years (4.4%) and more than 15 years (50.0%). Homozygous AA (73.08%) individuals had a parasitaemia> 1000 trp/µL while heterozygotes AS (7.14%) had a parasitaemia <1000trp/µL, 100% of SC had a parasitaemia<1000 trp/µL as well as 30.30% of AC. The most common blood groups were O + (46.15%) and B + (24.72%). **Conclusion**: The low prevalence of parasitaemia observed in individuals with sickle cell traits (HbAS, HbAC and HbCC) and major sickle cell syndrome (HbSC) would confirm the hypothesis of protection against asymptomatic malaria infection in Burkina Faso.

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INTRODUCTION

Malaria is a parasitic disease caused by a protozoan of the genus Plasmodium which alternately infects human hosts and mosquitoes. *Plasmodium falciparum*, the species that causes the deadliest form of malaria, is widespread by *Anopheles gambiae* and *Anopheles funestus* (Crompton *et al*., 2010). Malaria is a major cause of morbidity and mortality in tropical regions. According to the WHO, *Plasmodium falciparum* is the main cause of 219 million cases of malaria morbidity and 435,000 cases of death due to malaria worldwide in 2017. Africa accounts for more than 90% of these cases with a significant proportion (61%) of children under the age of 5(WHO, 2018). In Burkina Faso, malaria remains a stable endemic disease throughout the country, with a seasonal peak from May to October. It was the leading cause of consultation (46.50%), hospitalization (61.50%) and death (30.50%) 2013 according to statistical data from the National Malaria Programme (PNLP, 2014, Douamba *et al*., 2012, Douamba *et al*., 2014). Indeed, the country has recorded more than seven million cases of simple malaria in all health facilities, with 414,234 cases of severe malaria and more than six thousand deaths (Health, 2014). Malaria pathogenesis is determined by several factors that are characterized by the interactions between the responsible parasite and the genetic susceptibility and/or immune system of the host (Labie, 1997). The severity of malaria also depends on the parasite's ability to interact with the host's red blood cells. Some individuals with sickle cell trait (HbS and HbC) are considered to be resistant to malaria (Verra *et al*., 2009).

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Studies related to protection against malaria by hemoglobin S and C are still controvesal (Verra *et al*., 2009).The objective of this study was to assess the involvement of hemoglobin S and C in the protection against asymptomatic *Plasmodium falciparum* infections in Burkina Faso.

MATERIALS AND METHODS

Study site and population: The population of this study is consisted of 83 children aged 0 to 59 months and 99 adults. They were enrolled in the care and social promotion centre (CSPS) in the rural commune of Koubri. In this area located 30 km outside of Ouagadougou, malaria is endemic with an annual entomological inoculation rate of 441.6(Hay *et al*., 2000). Its climate is of the Sudanese type with a dry season from November to May corresponding to the period of low malaria transmission and a rainy season from June to October corresponding to the period of high transmission. Agricultural activities are carried out mainly during the rainy season and gardening during the dry season around artificial reservoirs. These activities promote the presence of Culicidea throughout the year including *Anopheles gambiaesl* main vector which thus permanently maintains the transmission of malaria caused by *Plasmodium falciparum* (Petrarca *et al*., 1986).

Blood samples: The samples were taken from September to November 2012. A volume of 4 mL of venous blood was taken from an EDTA tube and transported to the Pietro Annigoni Biomolecular Research Center (CERBA) using a refrigerated cooler for carrying out the biological tests.

Determination of parasitaemia: The thick-drop and blood smear slides were made at the time of collection of blood samples using the WHO technique(WHO, 1995). The coloration of slides was performed at Giemsa 10% according to the technique described in the WHO protocol (WHO, 1995). The slides were then examined under an optical microscope. The parasitic density was calculated using the following formula:

PD (trp/µL of blood) = (Y x 8000)/X

PD: parasitic density; **Y**: number of parasite counts counted; **X:** number of leukocytes counted. The count was carried out based on 1000 leukocytes and stopped after 500 parasites counted even if the number of 1000 leukocytes was not reached.

Complete blood count: The blood sample taken from EDTA tube was mixed slowly for 10 minutes on a KJMR-W mixer before being analyzed using the Micros 60 ABX hematology meter.

ABO blood Group and Rhesus Factor: The determination of the blood group was carried out using Beth Vencent's technique. A drop of blood was mixed with a drop of each anti-serum (Anti-A, Anti-B, Anti-AB and Anti-D) previously deposited on an opaline plate. The plate then undergoes some manual rotations movements or on a RM-500 mixer from Hospitex Diagnostics to facilitate antibody-antigen complexation.

Hemoglobin electrophoresis: The hemoglobin electrophoresis was performed using the Helena Bioscience electrophoresis chain, comprising a plate (SAS1) for electrophoretic migration and a gel staining tank (SAS2). It consisted first in preparing the blood sample by carrying out 3 steps of washing the hemoglobin with a physiological 0.9% sodium chloride solution followed by the lysis of the hemoglobin by a hemolyser. After this first step, electrophoretic migration is carried out to separate the fractions of the hemoglobin on agarose gel. The identification was made in the presence of reference samples (HbAA, HbAS, HbAC, HbSC, HbSS, HbCC). At the end of the migration, the gel was dried and then colored at acid or alkaline pH before reading with a densitometer.

Ethical consideration: This study has received the approval of the national ethics committee in health science of Burkina Faso (Deliberation No. 55/MS/MESSRS/CERS). The free and informed consents were obtained from adult participants or guardians of individual under 18 years of age prior to their enrollment in the study.

Statistical analysis: The Statistical Package for Social Sciences (SPSS) software version 21.0 and EPI-info version 6.04 dfr (CDC, Atlanta, USA) were used respectively for descriptive statistics and comparison of proportions. The differences were considered significant for $p<0.05$.

RESULTS

Socio-anthropological and clinical characteristics of the study population: The present study focused on a population of 182 participants which is characterized by children under 5 years of age (45.6%), adolescents (4.4%) and adults over 18 years of age (50.0%). The female (55.0%) was more represented than the male. Parasite densities (Pd) ranged from 40 to 180,300 trp/µl of blood and were higher in children who had p> 10,000 trp/µl compared to adults (0.52%) with the same density. The mean hemoglobin level was 10.97 ± 2.24 g/dl. The prevalence observed for the hemoglobin genotypes was: 73.08% for homozygous AA, 18.13% for AC and 7.14% for AS. The double heterozygote SC had a prevalence of 1.10% and the homozygote CC had a prevalence 0.55% (Table 1). The blood groups most frequently found were the O rhesus + (46.15%) and B rhesus + (24.72%) (Table 2). Table 3 shows the influence of hemoglobin genotypes on *P. falciparum* parasitaemia. When the parasitaemia is greater than 1000 trp/mL, the prevalence of individuals HbAC (9.09%) and HbAS (7.69%) was lower than that of HbAA (15.04%); this means that these types of sickle cell traits develop less severe malaria than HbAA (Table 3). The prevalence of malaria-positive according to hemoglobin genotypes was higher in the HbAA genotype (25.82 %), followed by HbAC (7.14 %), HbAS (2.75 %) and HbSC (1, 10 %) (Fig. 1).

DISCUSSION

This present study involved 182 subjects (adults and children) from the rural commune of Koubri located in a malaria endemic area of Burkina Faso. The mean age was 20.14 ± 19.89 years ranging from 1 to 76 years. The mean hemoglobin level in this population was 10.91 ± 1.95 g/dL and ranged from 9.56 to 12.46 g/dL. The O reshus + profile was the most common blood group (46.15 %) and AA genotype was the most observed electrophoretic profile of hemoglobin (73.08 %). The objective of the study was to determine the involvement of hemoglobins S and C in the protection against asymptomatic *Plasmodium falciparum* infections in Burkina Faso.

Table 1. Socio-anthropological and clinical characteristics

Age (years)	Population		Hemoglobin levels (g/dL)		Parasitemia (trp/µL)		
	N	(%)	≤ 12.5	>12.5	0	≤ 1000	>1000
Sex-ratio	82/100	82	-	-			
≤ 5	83	45.6	81 (58.27%)	2 (4.65%)	43 (37.39%)	22 (51.16%)	18 (75.00%)
> 5	99	54.4	58 (41.73%)	41 (95.35%)	72 (62.61%)	21 (48.84%)	6 (25.00%)
Total	182	100	139	43	115	43	24
P value			0.027	< 0.0001	0.0001	0.829	0.0005

Table 2. Influence of Blood group on *P. Falciparum* parasitemia

Blood group/Rhesus	Population		Parasitemia (trp/µL)			Mean	Standard Deviation	p-value
	N	(%)	0	≤ 1000	>1000			
A+	36	19.78	27	7	2	724.38	2956.78	
AB-	1	0.55	1	0	0	0	-	
AB+	8	4.40	5	1	2	705	1318	
B-	4	2.20	2	1	1	4257.50	8250.48	NS
B+	45	24.72	29	7	9	1701.11	4379.08	
O-	4	2.20	1	2	1	1635	2979.60	
O+	84	46.15	50	25	9	3599.24	20705.70	

Table 3. Effect of hemoglobin genotype on *P. falciparum* parasitemia

Hemoglobin genotype	Population		Parasitology (trp/µL)		
	N	(%)	0	≤ 1000	>1000
AA	133	73.1	85/133 (63.91%)	28/133* (21.05%)	20/133* (15.04%)
AC	33	18.1	20/33 (60.61%)	10/33^ (30.30%)	3/33^(9.09%)
AS	13	7.1	9/13 (69.23%)	3/13° (23.08%)	1/13°(7.69%)
Cc	1	0.6	1/1	0	0
SC	2	1.1	0	2/2	0
Total	182	100	115	43	24
P value				* → ^: p = 0.257 * → °: p = 0.853 ^ → °: p = 0.853	* → ^: p = 0.546 * →°: p = 0.759 ^ → °: p = 0.667

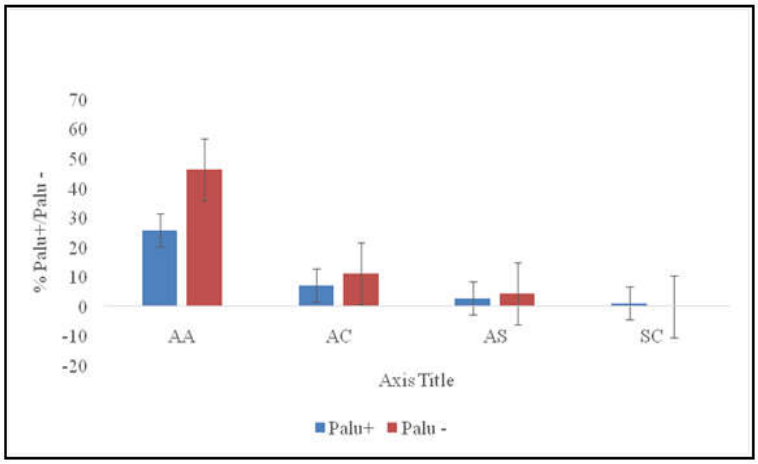

Figure 1. Frequency of malaria by hemoglobin genotype

The the distribution of genotypes in the present study population is comparable to that observed by Chillemi *et al.* (Chillemi *et al.*, 2004) and Ouattara *et al.* (Ouattara *et al.*, 2016). Table 3 shows that the homozygous AA individuals were the most common group with parasitaemia greater than 1000 trp/µL (p = 0.0005). Similar results were also observed by Amodu on a population of 3,100 children in Nigeria in 2012 (Aluoch, 1997, Modiano *et al.*, 2001, Amodu *et al.*, 2012). In the present study population, no statistically significant difference was found between the distribution of parasitemia according to ABO system. However, the mean parasitic densities above 1000 trp/µL were observed in groups B- (4257.50 trp/µL), O+ (3599.24 trp/µL), B+ (1701.1 trp/µL) and O- (1635 trp/µL) (Table 2). Due to the small size of the present study population, no homozygous SS individual was reported unlike some previous studies (Modiano *et al.*, 2001, Cyrklaff *et al.*, 2011) (Table 3). The low prevalence of parasitemia found in HbAS, HbAC, HbSC and HbCC individuals (p-lt; 0.05) supports the hypothesis of protection hbS and HbC alleles against asymptomatic malaria infection (Table 3). Indeed, numerous association studies have shown that the hemoglobin alleles S and C do not provide protection against Plasmodium infection but rather against the progression of the disease toservere forms (Lelliott *et al.*, 2015, Amoako *et al.*, 2014, Modiano *et al.*, 2001, Verra *et al.*, 2007).

Luzzatto had found that the parasitized AS hemoglobin had a sickling speed 2 to 3 times faster than the uninfected AS hemoglobin. Which would cause a programed death for infected HbAS (Labie, 2010).

Conclusion

The results of the present study showed a higherparasitemia in HbAA individuals and lower parasitemia in individuals with sickle cell trait that would provide protection against asymptomatic malaria infection in populations living in malaria endemic areas of Burkina Faso.

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