Original Article

Distribution of erythrocyte binding antigen 175 (EBA-175) alleles and ABO blood groups in a hypoendemic area in Senegal

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Abstract

Introduction: The study was conducted to determine for the first time the association between the erythrocyte binding antigen 175 (EBA-175) alleles and ABO blood groups in malaria patients living in Thies, a hypoendemic area in Senegal.

Methodology: In 2007, the EBA-175 alleles and blood group types were determined by nested PCR and the Simonin test respectively in blood samples obtained from uncomplicated *Plasmodium falciparum* malaria positive patients.

Results and conclusion: In total, 129 patients were enrolled in the study. The EBA-175 genotyping showed a prevalence of 67.45% for the F-allele, 27.90% for the C-allele and 4.65% of mixed C+F infection. The distribution of the ABO blood group type showed 59.8% for the O group, 19.7% for the A group, 17.2% for the B group, and 3.3% for the AB group. No correlation was noted between the EBA-175 alleles and either the blood group type or parasitemia.

Key words: *Plasmodium falciparum*; EBA-175; blood groups; Thies; Senegal

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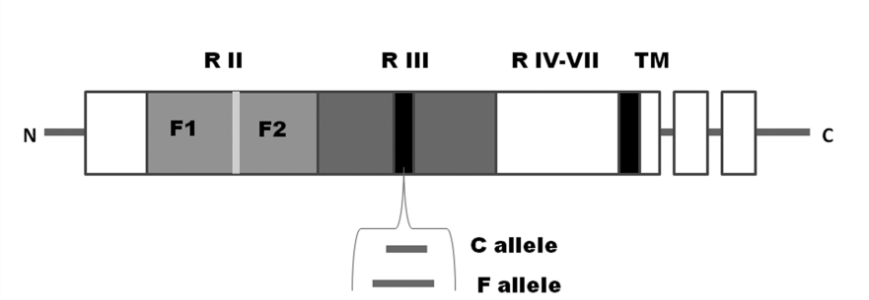
Introduction

Despite the enormous efforts in the fight against malaria through the tentative development, of vaccines or new drugs, the disease is still a public health concern. The parasite's life cycle is complex both in vertebrate hosts and *Anopheles*. In humans there is an asymptomatic pre-erythrocyte stage followed by an erythrocytic phase, which is responsible for the clinical symptoms. This step begins with the invasion of red blood cells by the merozoites, which is a rapid process governed by molecular interactions between the merozoite and the red blood cell. This interaction involves parasite ligands and receptors on the erythrocyte surface and several pathways have been identified as being used during the invasion process. The main sialic acid dependent pathway uses EBA-175 in the merozoite and glycoprhorin A in the red blood cell. EBA-175 is divided into seven regions classified from I to VII [1] (Figure 1). Region III of EBA-175 contains either F (FCR3 strain) or C (Camp strain) segments that define dimorphic allelic family sequences [2].

The major difference between the two dimorphic proteins is the presence of a small defined set of amino acid residues. The Camp EBA-175 protein contains a 113-amino-acid-residue domain called the C segment, and the FCR-3 EBA-175 protein a 139-residue domain designated as the F segment [2]. These domains are also involved in the interaction between the merozoite and the red blood cell. Although they do not possess significant sequence homology, when expressed *in vitro*, they both bind to erythrocytes in a sialic acid-independent manner and contain cross-reactive epitopes [3].

Thus it is very important to study the interaction between merozoites and red blood cells. In clinical practice, the ABO system is best known for blood compatibility studies and has been the subject of several investigations of its association with infectious and non-communicable diseases [4,5]. The

Figure 1. Erythrocyte binding 175 (EBA-175) domains



relationships between blood group distribution and malaria, and between ABO and malaria, have been suggested for decades [6].

Studies have shown that group O subjects have protection against severe malaria [7-9].

A study in Sri Lanka showed strong statistical evidence of an association between ABO and disease severity in *Plasmodium falciparum* infection [10]. It has been shown that *Plasmodium falciparum* form larger, stronger rosettes in non O blood groups (A, B, AB) than in group O erythrocytes [11]. Interestingly, some strains of *P falciparum* preferentially trigger rosette formation depending on the red blood group [12].

Furthermore, an especially high prevalence of group O coupled with a low prevalence of group A is found throughout sub-Saharan Africa, where *P. falciparum* persists to this day. It appears that the A and B antigens are receptors for rosetting on uninfected red blood cells [13], because they are bound by a parasite protein called PfEMP1 which is expressed on the surface of infected red blood cells [14]. Rosettes still form in group O red blood cells (albeit smaller and weaker than those that form in non-O red blood cells) through the involvement of other red blood cell molecules which act as alternative receptors for rosetting. Consequently, the contribution of the ABO blood group system to malaria infection must be investigated further.

Our goal was to determine for the first time the distribution of EBA-175 alleles and ABO blood group in Thies, a hypoendemic area in Senegal.

Methodology

Patients

Patients were recruited in Thies, Senegal, a hypoendemic area located 70 kilometers from Dakar. The study was approved by the IRB of the Harvard School of Public Health and the Comite d'ethique du Senegal.

Samples were obtained from patients with uncomplicated malaria during the 2007 transmission season (October to December). Informed consent was obtained from the participants and parents or guardians of all participating children. All participants monitored were screened for *Plasmodium falciparum* infection by light microscopy. Blood was collected by venipuncture using EDTA Vacutainer tubes Tyco Healthcare Group Lp, Mansfield, USA) for the determination of blood groups. Blood was spotted on Whatman filter paper and dried at room temperature for the extraction of *P. falciparum* DNA. Filter papers were sealed in plastic bags with silica gel until DNA extraction. Thick and thin blood films were Giemsa-stained and examined with a light microscope, and parasitemia was determined as percentage of infected cells. Socio-demographics and clinical data such as age, sex, parasitemia, axillary temperature, hemoglobin level, and hematocrit were also recorded. All participants diagnosed with *P. falciparum* malaria were treated based on the National Malaria Program recommendations. The study population was divided into two groups: under 10 years, and over 10 years of age.

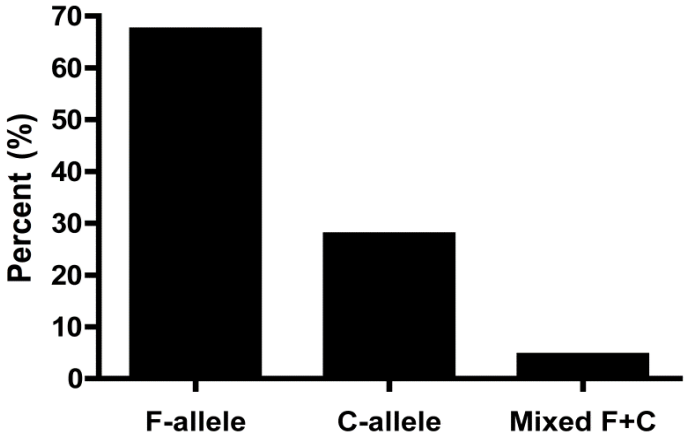
DNA extraction

Parasite DNA was extracted from dried blood spots by using the QIAmp DNA mini kit (Quiagen, Hilden Germany) by following the manufacturer's instructions.

EBA-175 genotyping

The typing was performed by nested PCR as described elsewhere [15]. The outcome of the

Figure 2. Distribution of EBA-175 alleles



amplification was controlled by electrophoresis in an ethidium bromide-stained 1% agarose gel. For detection of the stained DNA, an ultraviolet transluminator and specific detection software was used. A PCR product at 795 bp indicated an infection with the F-fragment while a band at 714 bp showed an infection with the C-fragment. The presence of two bands at 795 and 714 respectively indicated a mixed infection of the patient with at least two different parasite clones.

Blood group typing

Blood groups were determined using the Simonin method, which is an erythrocyte test for identifying circulating plasmatic antibodies. The patient's serum or plasma containing the potential circulating antibodies was mixed with A red blood cells and B red blood cells and agglutination was recorded. The blood group was determined by lack of agglutination (a lack of antibodies to that blood group).

Statistical analyses

Analyses were performed using statistical packages SPSS 16.0 (IBM, Chicago, USA) and STATA 11.0 (StataCorp, College Station, TX, USA). Statistical Student's T test was considered significant if P values were < 0.05.

Results

Frequencies of EBA-175 genotypes and ABO blood groups in the population

A total of 129 patients were enrolled in our study, and 38% of them were female. The majority of the study population (85%) was older than 10 years of age. Parasitemia varied between 0.1% to 12.5% with a mean of 2.2 ± 0.4. A significant negative correlation was found between age and parasitemia ($P = 0.011$). The EBA-175 genotyping showed that 67.5% of the 129 patients carried the F-fragment, 27.9% carried the C-fragment, and 4.6% carried C-F mixed infections (Figure 2). As shown in Figure 3, among the 122 participants whose blood types were determined, the O group was the most prevalent (59.8%), followed by the A group (19.7%), the B group (17.2%) and the AB group (3.3%).

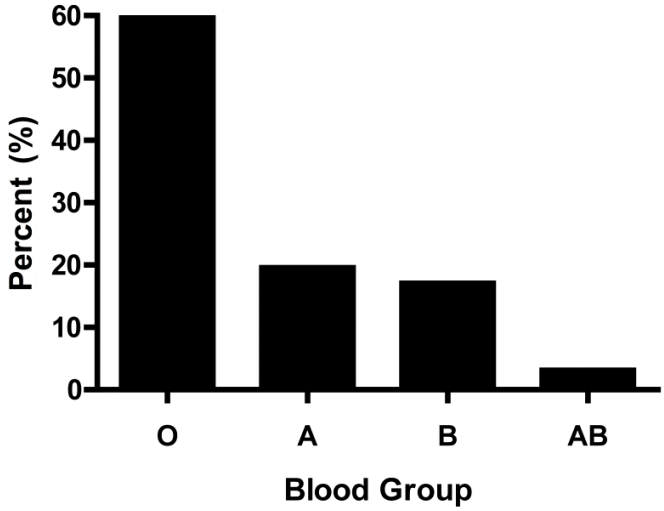
EBA-175 alleles and blood groups

In patients with blood group O, we observed the following frequencies of EBA-175 alleles: 67% F-fragment, 27% C-fragment and 6% mixed infection (F-C). For patients with blood group A, we observed 63% F-fragment and 37% C-fragment and no mixed infections. In patients with blood group B we observed 71% F-fragment, 19% C-fragment and 10% mixed infection. In the AB blood group we observed 75% F-fragment, 25% C-fragment and no mixed infections (Figure 4).

EBA-175 alleles and age

The F-fragment was predominant in both age groups, at 80% for patients under 10 years of age and 65.14% for participants 10 years or older. Mixed infections with the presence of both alleles were noted only in the age group younger than 10 years, but there were only six samples (5.5%. There was no correlation between the allele distribution and age group.

Figure 3. Blood group distribution



EBA-175 alleles and temperature, parasitemia, sex

Axillary temperature was measured for all patients and ranged from 37°C to 42°C (38.8°C ± 0.3°C).

There was no correlation between parasitemia and temperature. Similarly there were no correlations between EBA-175 alleles and age, sex or parasitemia.

Discussion

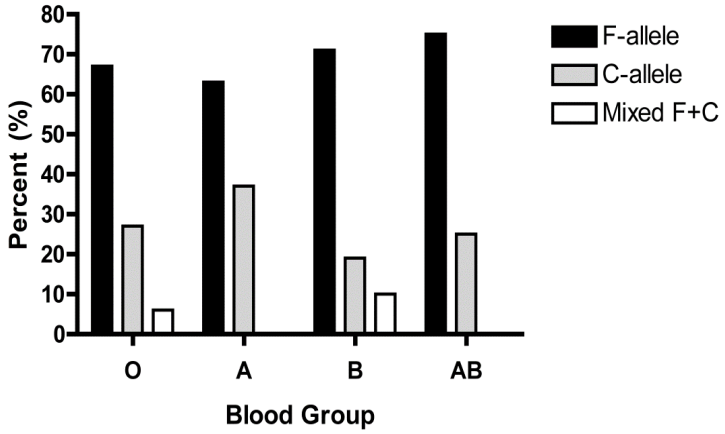
The invasion of erythrocytes by malarial merozoites is a complex process [16,17] that involves multiple steps and interactions with ligand receptors. Erythrocyte binding antigen 175 (EBA-175) of *P. falciparum* is one of the key components during the fast cascade of interactions between the parasite and host molecules before the merozoite completely invades the erythrocytes [18]. To detect the alleles of EBA 175 (F-fragment and C-fragment) and to map their distribution in Thies, a nested PCR method was used.

The results showed the presence of the two alleles with a predominance of the F-fragment (67.45%). Similarly, in Bakoumba, Gabon, the F-fragment was more prevalent in children [19]. Similar frequencies were also described in Nigeria, The Gambia, Gabon, and South Africa [20]. Outside of Africa, the F-fragment was also predominant in Northern Laos [21] and in Iran [22]. However, in Sudan, the C-fragment was the dominant allele in the population [20]. In Southern Laos, a study showed an almost equal distribution of the two fragments [21]. Surprisingly, our findings show a higher prevalence of the F allele which is different from observations previously described in two studies in Senegal [23,24]. This discrepancy might be attributed to the fact that our study site is a low endemic area, compared to those other sites (Velingara and Kaolack). A study conducted in Ghana in children with severe malaria showed a predominance of the F-fragment and it was noted that the C-segment was significantly associated with fatal outcome [25]. Our results taken together with these studies demonstrate that the distribution of EBA-175 alleles is different across geographic regions. According to Binks *et al*., genetic differences in the human host population may be a reason for allelic selection [20], while Okenu *et al*. have shown that antibodies against C or F alleles can be heterologous or homologous [26].

The interaction between the merozoite and the red blood cell is essential for invasion. While some ligand/receptor pairs have been identified, many remain to be discovered [27,28].

The ABO blood group system is arguably the best known blood group system on the erythrocyte, and yet the most functionally mysterious genetic polymorphism in humans. The relationship between the ABO system and diseases has been studied for many years, especially the association between malaria and ABO [29], because of the critical interaction between the *Plasmodium* parasite and the red blood cells.

Figure 4. Blood group type and EBA-175 alleles



In our study, subjects with blood group O represented 59.8% of the population, which is the case in most countries in the western hemisphere, where the distribution of group A and Group O generally matches malaria's tropical distribution [30]. In fact, in the general population in Senegal, we note a prevalence of group O of 50% against 25% for the group A (Badiane *et al*. unpublished data).

It is believed that the distribution of the ABO blood system alleles in Africa was influenced by *Plasmodium* selective pressure [30,31]. The distribution of blood groups shows that group O is relatively more represented in countries where malaria is prevalent. In regions where the disease is or was endemic, blood group O represents the majority type, such as in Southeast Nigeria (87.60 %), Kenya/Kiyuki (60%), and Central America/Amazon basin (90%). In contrast, in areas without malaria, such as Sweden (62%), Switzerland (60%), the Czech Republic (70%), and Portugal (65%), the non-O blood groups represent the majority. In Asia, the prevalence of group O rises among people who live closer to the equator. For example, in China, the prevalence for group O is 29% in Beijing (a cold weather zone), but it is 46% in Canton, which is a more tropical zone [30]. Many studies have focused on the association between malaria and blood groups, and have shown that individuals who belong to group O are protected against severe malaria [32,33,11,34-37]. Thus if survival from malaria is associated with group O, then the worldwide distribution of ABO groups is consistent with malaria selective pressure.

The repartition of the EBA-175 alleles according to blood group showed a predominance of the F-segment in each blood group type. Mixed infections were noted in the groups O and B. In the AB group, no mixed infections was noted, but the low representation of the AB blood group may be the reason (< 5%, n = 4). Our study population was composed of uncomplicated malaria patients, making it impossible to determine a relation between EBA-175 alleles, blood groups, and the severity of the infection. There were no correlations between any of the two EBA-175 alleles and sex, parasitemia, temperature, or age.

Our results showed that parasitemia is negatively correlated with age ($P = 0.011$, $P < 0.05$). In endemic areas, most individuals develop an immune response that controls the parasite replication but does not eliminate the parasite from blood. Numerous epidemiological studies conducted in areas of stable malaria transmission report an age-dependent increase in *Plasmodium*-specific immune responses, as well as an age-related decrease in malaria-dependent morbidity [38]. This immunity is naturally acquired and is (i) effective in adults after uninterrupted lifelong heavy exposure; (ii) lost upon cessation of exposure; (iii) species specific; (iv) somewhat stage specific; and (v) acquired at a rate which is dependent upon the degree of exposure [39-42].

EBA-175 is an important ligand interacting with the sialic acid of glycophorin A and permits the parasite to invade the red blood cell. The interaction between the *Plasmodium* parasite and the erythrocyte involves region RIII, and the role of this dimorphism in the host-parasite interactions, such as the difference in efficiency of red blood cell invasion related to genotype, remains unclear. Understanding the

mechanisms involved in this step can lead to strategies to control malaria.

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