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Full Length Research paper

A study of the distribution of cerebral malaria in Sudanese population

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The study was carried out to investigate the distribution of cerebral malaria in central region and to identify ICAM-1 alleles and genotypes frequency in Sudanese population in the region. Fifty children with cerebral malaria and 50 age- and sex-matched healthy controls with no history of cerebral malaria were enrolled in the study. The highest incidencts of cerebral malaria were found between the ages of 4–8 years. From 10 different tribal stocks, 28% of incidents belongs to the Johayna tribe in the western region of central Sudan. The incidence of cerebral malaria in this study is influenced by geographical, age, and ethnic factors with no gender variation. From this study four (8%) of study subjects died, 4 (8%) survived with neurological sequelae and 42 (84%) were discharged alive and healthy after treatment regimen. The use of allele specific PCR (ASP) for genetic analysis in this study, indicated incidence of the heterozygous form (K29/M29) is 26% in cerebral malaria patients and 12% in the control group, while only one (2%) mutant homozygous (M29/M29) was detected in cerebral malaria patients group. All subjects who carried mutant allele (heterozygous and homozygous mutant) had 3 times susceptibility to cerebral malaria than the other group, *(P-value= 0.038*, Odd Ratio = 2.5; 95% CI 1.011 - 6.181). The incidence of ICAM-1^{kilifi} allele frequency in the study group was 11%, and this may increase the risk for susceptibility to cerebral malaria in Sudanese children inhabiting in these regions.

Key words: ICAM-1; cerebral malaria; children; central Sudan.

INTRODUCTION

Malaria continues to be a major health problem in many parts of the world. Over 2400 million people at the risk of infection (WHO, 2000). Mortality from malaria in African children under 5 years is 36/1000 per year (Barnish et al., 1993). The patterns of pathology differ with changes in the degree of endemicity (Miller et al., 1994). Cerebral malaria (CM) is the main causes of death in children and non immune adults (Engwerda et al., 2002).

Malaria incidence in Sudan was estimated to be about 9 million episodes in 2002 and the number of deaths due to malaria was about 44,000 (Abdalla et al., 2007).

Malaria risk is present all over the country although too different degrees. In the northern, eastern, and western states, malaria is mainly low to moderate with predominately seasonal transmission and epidemic outbreaks. In southern and central Sudan, malaria is moderate to high or highly intense generally with perennial transmission. *Plasmodium falciparum* (*P. falciparum*) is the dominant parasite and the principal mosquito vectors are *Anopheles arabiensis*, *A. gambiae* and *A. funestus* (Malik et al., 2004). Ninety percent (90%) of all malaria attacks are caused by *P. falciparum*, which causes the most severe form of disease and deaths attributable to malaria in Sudan (AL Gadal 1990). High seasonal rainfall, variation in temperature and humidity

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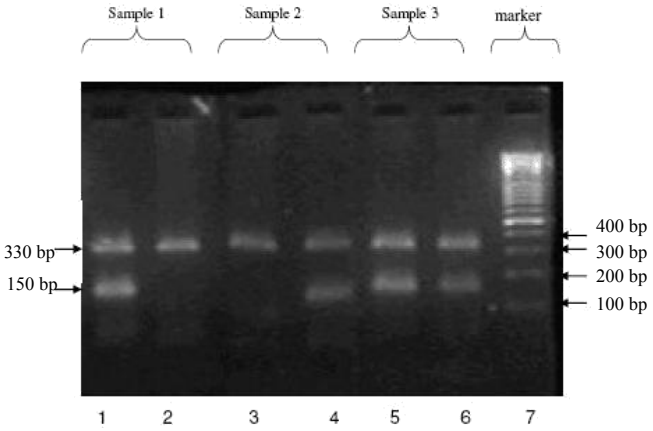

Figure 1. Detection of ICAM-1 polymorphism using ASP method. Lane 1 and 2 are sample 1 (Wild type); Lane 3 and 4 are sample 2 (homozygous mutant); Lane 5 and

that is observed during the rainy season in central Sudan can favor mosquito breeding resulting in strong seasonal transmission.

The virulence of *P. falciparum* is thought to be due to the adherence of parasitised red blood cells to small vessel endothelium through several receptors, including CD36, thrombospondin, and intercellular adhesion molecule-1(ICAM- 1). Binding ability varies between different parasite isolates moreover as well as being receptor for parasite adhesion, ICAM- 1 (CD54) plays a major role in normal immuno-function mechanisms (Stolpe 1996). Immunohistochemical studies have implicated ICAM-1 as of potential importance in the pathogenesis of cerebral malaria. A mutation at position 29 was found in different racial groups and the ICAM-1 polymorphism from samples from a case–control study indicated an association of the polymorphism with the severity of clinical malaria. Individuals homozygous for the mutation have increased susceptibility to cerebral malaria (Fernandez-Reyer et al., 1997; Kun et al., 1999).

PATIENTS AND METHODS

Study area

The study was carried out at the hospitals of Wad Medani, Sinnar, and Singa towns. These towns lie along the Blue Nile. Wad Medani lies about 184 kilometers South to Khartoum, capital of Sudan, within the largest Gezira agricultural scheme. Sinnar lies down Sinnar Dam, which irrigates Gezira agricultural scheme, while Singa lies about 120 kilometers down Damazin Dam which lies near Damazin town. Thus, the vector, anopheles' mosquito is present throughout the year in these areas endemic to *P. falciparum* during the rainy season.

Study design

In the present hospital based study, patients with asexual falciparum malaria who had unrousable coma that persisted for more than 1 hour after convulsion and not attributed to any other causes were recruited from the three hospitals. Fifty positively diagnosed cerebral malaria children; 18 (36%) from Wad Medani Pediatric Teaching Hospital, 27 (54%) from Sinnar hospital, 5 (10%) from Singa hospital were enrolled in the study. All patients with blood film negative for asexual stages or having other diseases that may had contributed to the same complication of CM were excluded from the study. Fifty age and sex matched children were selected as a control from different schools in the three cities, or from patients from the three hospitals who were admitted for other diseases than CM and have no history for CM.

Genotype of ICAM-1 variants using PCR-ASP

Allele specific PCR (ASP)

Allele specific PCR also called the amplification refractory mutation system (ARMS) was used (Newton et al.; 1989; Wu et al., 1989). In this method, two separate and complementary PCR amplifications for each sample, one was specific for ICAM-1^{Kilifi} allele and the other for ICAM-1^{ref} allele, using three primers were done. One was common for the two reactions, and other two, one for each reaction, which differs from each other at their 3' ends, specific for the particular variant base (A/T) .

PCR reactions

The total volume of the PCR reactions was 30µl, containing 4µl genomic DNA, 10 picomoles for each primer of the two allele, 1picomole for internal control primers, 200µM dNTPs (dATP, dGTP, dCTP, and dTTP), 2.5µl from 10 x Taq Gold Buffer (100mM Tris HCl, pH 8.3, 500 mM KCl, 15 mM MgCl2 and 0.01 % (w/v) gelatin (Perkin Elmer Cetus), 2 U AmpliTaq GoldTM polymerase (Perkin Elmer Cetus), and completed to the total volume with deionized water. 150bps of DNA fragment was PCR amplified using designed primers; primer1 common for both reactions, primer2 for ICAM-1^{kilifi} reaction only, and primer3 for ICAM-1^{ref} reaction only, The primer1 was 5'TGCCTGTCGCCTCTTCCCT3'; primer2 was GGTCTCTATGCCCAACAAC A and primer3 was GGTCTCTATGCCCAACAAC T. 330 bps of internal control was also amplified. The PCR condition was; 94°C as initial denaturation for 3 minutes, followed by 35 cycles of 94°C as melting temperature for 45 minutes, 64°C annealing for 45 minutes, and 72 as extension for 45 minutes. Then a final step in 72°C for 3 minutes as a final prolongation. 10µl of PCR product electrophoresed at 100V for 15 minutes in 2% agarose gel in 0.5xTBE (Tris base boric acid EDTA) buffer (running buffer), loaded with 5µl bromo phenol blue (loading buffer). The gel and running buffer contained ethidium bromide (0.5µg/ml), which stained the DNA before placed the gel on a UV light for visualization and finally photographed.

ICAM-1^{ref} homozygous (Wild type- K29/K29) is characterized by the amplification produced only in the ICAM-1^{ref} reaction, a heterozygote (K29/M29) characterized by the amplification produced in the both reactions and ICAM-1^{Kilifi} homozygous (Homozygous Mutant- M29/M29) characterized by the amplification produced only in the ICAM-1^{Kilifi} reaction (Figure-1).

Statistical analysis

Statistical analyses were performed with the SPSS (version13.0) package. Data were expressed as mean ± SD, Frequencies. ICAM-1 genotype was calculated by counting the allelic pair composition of the gene. Allele frequencies were determined by counting the number of chromosomes bearing an allele. Association between genotypes and CM or the other parameters were assessed using

Table 1. The genotype frequencies of ICAM-1 in CM patients and control (n = 50).

ICAM-1	Genotypes		
Group	K29/K29	K29/M29	M29/M29
CM Patients	36(72%)	13(26%)	1(2%)
Control	44(88%)	6(12%)	0(0%)

Table 2. The allele frequencies of ICAM-1 in CM patients and control.

ICAM-1	Genotypes		Alleles	
Group	K29/K29	K29/M29+ M29/M29	K29	M29
CM Patients	36(72%)	14(28%)	0.85	0.15
Control	44(88%)	6(12%)	0.94	0.06
P -values	0.046		0.038	

(Odd Ratio = 2.5; 95% CI 1.011 - 6.181).

Table 3. The distribution of ICAM-1 genotypes in CM subjects according to the outcome phenotypes.

ICAM-1	Genotypes		
CM outcome	**K29/K29**	**K29/M29**	**M29/M29**
Dead and neurological sequelae	6(75%)	2(25%)	0(0%)
Discharged alive and healthy	30(71.4%)	11(26.2%)	1(2.4%)

P-value = 0.902.

chi square and Odds Ratio with confidence intervals (CI 95%) which are interpreted as the relative risk of disease for "exposed" compared with "unexposed" persons. P 0.05 was taken as the level of significance.

RESULTS

The distribution of study subjects has shown that Sinnar hospital had the highest incidence of CM compared to the other two hospitals 54% (n =27). The age of children diagnosed with cerebral malaria ranged between 2 and 14 years with a mean of 7.29 +/- 3.5. The highest incidence of disease was at the age group 4-8 years. Fifty six percent were males, 44% were females, and there was no significant difference between the genders *(P=0.71)*. The study subjects extended along 10 different tribal stocks, 68% of them belong to four tribal stocks, (28%) belong to Johayna tribal stock and (16%) to Gaalian tribal stock, while (12%) belong to Nuba and Nigerian tribal stocks.

Four (8%) of the children died and four (8%) survived with neurological sequelae (Hemiparesis, Hemiplagia, Aphasia, and Quadriparesis and Blindness) while forty - two (84%) were discharged alive and healthy after treatment.

ICAM-1 genotype screened revealed that the wild type (K29/K29) was 72% in CM patients and 88% in their control, the heterozygous (K29/M29) was 26% in CM patients and 12% in the control group, while only one (2%) mutant homozygous (M29/M29) was detected in CM patients group, but was not found in the control group (Table 1). There was no significant difference in the distribution of ICAM-1 genotypes, but the difference was found between the wild type and the carrier to mutant allele (heterozygous and homozygous mutant) with *P-value= 0.046.* The allele frequencies in the control group (n=50) were 0.94 for (K29), and 0.06 for (M29), and in CM subjects (n=50) were 0.85 for (K29), and 0.15 for (M29). The distribution of alleles between subjects and control has shown significant difference, and all subjects who carried mutant allele had 3 times susceptibility to CM than the other group (P- value= *0.038*, Odd Ratio = 2.5; 95% CI 1.011 - 6.181) (Table 2). The distribution of ICAM-1 genotypes according to CM outcome, there was no association observed between them (P-value 0.902; Table 3).

DISCUSSION

The distribution of the study subjects has shown that Sinnar hospital had the highest incidence of CM and the age showing the highest incidence of the disease from 4 – 8 years and no difference was observed between male

and female. This might be attributed to the low immunity of these children at this age. Johayna tribal stock from western region of central Sudan had CM clustering (28%), while Beja, Nilotic, and Moroccan and Egyptian had the less clustering (2%). Confirming the distribution of the disease influences by geographic, age, and ethnic factors. The mortality and morbidity rate were high 8% of children died and other 8% survived with neurological sequelae (hemiparesis, hemiplagia, aphasia, quadriparesis and blindness). Recently a mutation that caused A to T transversion in the ICAM-1 gene, causing a Lys to Met change in the amino acid sequence defined as a potential importance in the pathogenesis of cerebral malaria (Mcguire et al., 1996; Fernandez-Reyer et al., 1997). The distribution of this mutation was found in different populations in Africa and North America. In the North American population, it was found predominantly in African-Americans (41%) (Zimmerman 1998). No Caucasian was found to carry the ICAM-1 gene mutation (Fernandez-Reyer et al., 1997) . In a two Asian populations studied in Papa New Guinea and Thailand, only in the Papa New Guinea was the ICAM-1 gene mutation found. Thai did not carry the mutation. In contrast this mutation was found in Sudanese populations as shown in our study in a frequency of (20%), 19% (n =13) were heterozygous, 1% (n =1) were homozygous. This is in agreement with studies from Kenya, Nigeria, Gabon, and Papa New Guinea (Fernandez -Reyer et al., 1997; Mcguire et al., 1996; Kun et al., 1999) which indicate that this mutation is predominant in populations residing in malaria endemic areas however. However the current study showed less frequency when compared to the study done in Kenya where the frequency reached up to 60% (47% heterozygous and 13.59% homozygous) (Kun et al., 1999). The results obtained from this study suggested that group carrier to this mutation is highly susceptible to CM than the other group (P = 0.046). This is in agreement with a study done in Kenyan children which found this mutation had a high susceptibility to cerebral malaria. In a study carried in the Gambian children (Mcguire, et al., 1996), no correlation between ICAM-1 gene polymorphism and disease severity was found. Other study in Gabonese children (Mcguire, et al., 1996), found this mutation protects against severe malaria.

The discrepancies between these studies can be attributed to geographic differences of *P. falciparum* strains that bind with different affinities to endothelial cells with this mutation. The Gabon study (Mcguire, et al., 1996; Kun, et al., 1999) compared severe malaria patients with those with mild uncomplicated malaria and the incidence of CM was rather low (n = 9), in addition, three were heterozygous and six were wild type. In that study, influence of ICAM-1 might be by other genetic factors in other group of severe malaria than CM. The presence of this mutation at a high frequency in malaria endemic areas in Africa can confer a protective effect to pathogens other than *P. falciparum*, such as bacterial infections, etc

In conclusion, the African mutation (ICAM-$1^{kilifi)}$ is predominant in Sudanese population as 20% for carriers and 0.11 for allele frequency. This allele increases the risk for susceptibility to cerebral malaria three times than the other allele (P = 0.038, Odd Ratio = 2.5; 95% CI 1.011) . The study of this mutation with other pathogens is highly recommended.

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