Original Article

Antibodies against Merozoite Surface Protein 1 and 2 in Sudanese children

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Abstract:

Background: Malaria is a serious childhood disease causing high morbidity and mortality despite control measures. Immunological control against malaria was initiated early, and immunity acquired by children in endemic areas, which is age and exposure dependant, differs in different endemic settings.

The objective of the study was to determine antibodies against Merozoite surface protein-1 (MSP-I) and Merozoite surface protein-2 (MSP-2), and to determine their relation to the age of Sudanese children.

Methodology: The study was descriptive, cross-sectional, conducted in Khartoum Children Emergency Hospital (KCEH). 150 children with positive blood films for *P. falciparum* malaria were classified according to age, and a blood sample was taken from each one, and tested for antibodies against MSP-I and MSP-2.

Results: Antibodies to MSP-I and MSP-2 were 46% and 42% respectively. Sero-positivity and sero-negativity for both antigens were 26.6% and 42.7% respectively. Seropositivity to either MSP2 or MSP1antigen alone was present in 18.7% and 12% of patients respectively.

High seropositivity (52.9%) was found in the age group 12-15 years of age.

Conclusion: MSP-1 and MSP-2 antibodies in Sudanese children according to this study were age dependant, and findings were similar to what had been reported in some African countries.

Key words: *P. falciparum* malaria, MSP-I, MSP-2.

Malaria is an infectious disease that is associated with high morbidity (500 millions/year) and high mortality (1.5 – 1.7 millions/year) in spite of global effort exerted in control. Malaria mortality affects mainly children below five years of age (one million/year)[1] most of which is due to severe and complicated forms. Malaria causes a significant social and economic impact in developing countries by debilitating the active population[2]. According to WHO malaria is endemic in 99 countries, predominantly in Africa, Asia and Latin America[3].

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Immunological measures in malaria control were initiated in 1910[4], and in 1976 the United Nations Development Program (UNDP), the World Bank and WHO considered the development of malaria vaccine a top priority. More purified malaria parasites vaccines of merozoites, sporozoites and gametocytes were developed since then[5]. The most extensively investigated vaccines included Merozoite surface protein-1 (MSP-1) and Merozoite surface protein-2 (MSP-2), circumsporozoite protein-1(CSP-1) and ring infected erythrocyte surface antigen RESA[6].

During shizogony, *Plasmodium falciparum* asexual blood-stage synthesizes two major glycol-proteins, MSP-1 and MSP-2. These are membranes anchored via a glycosyl phosphatdylinositol GP-1[7]. MSP-1 is a potential malaria vaccine component. In the laboratory studies, antibodies to MSP-1 were found to inhibit parasite development in vitro in humans, probably by inhibiting protease

cleavage[8]. Epidemiological studies have shown that both anti-bodies and T-cells proliferation and cytokines response to MSP-1 and MSP-2 were associated with reduced susceptibility to clinical malaria.

Several studies suggested that anti-body's response to the dimorphic region of MSP-1 were more prevalent than response to the conserved region but may be short lived with seasonal variation[9]. The response to N-terminal MSP-1 regions was more prevalent than antibodies to MSP-1/42 region. Prevalence to MSP-1 antibodies in Gambian and New Guinea children were 50% and 90 respectively[10]. Low prevalence of antibodies in cross-sectional studies may therefore, reflect a dynamic situation in which many individuals develop anti-bodies but loose them shortly after each malaria attack.

In a study conducted in Kenya, antibody responses in infants against MSP-2 were low throughout their first year of life[11]. In another study done in the USA, mice immunized with MSP-142 were partially protected against *P. chabaudi* malaria, indicating a role for protective mechanisms of immunity[12].

Under these practical problems, studies on the immune response to malaria and the possible development of effective vaccines are of obvious interest and importance[13]. Perhaps the single most important factor which ameliorates the risk of a symptomatic infection proceeding to life threatening pathology is the development of clinical immunity[14].

The objectives of this study were to determine the ratio of antibodies against MSP-I and MSP-2, and to evaluate the relation of MSP-1 and MSP-2 to the age of Sudanese children.

Methodology:

Study design:

Descriptive, cross-sectional, hospital-based study of MSP-1 and MSP-2 in Sudanese children.

Setting:

The study was conducted during 2006, at Khartoum Children Emergency Hospital (KCEH), the biggest pediatric hospital in Sudan. The patients were derived from the city, the rural of Khartoum and all over the country.

Study population:

The study included all children, aged 2-15 years, who presented with clinical manifestations of malaria and confirmed by laboratory investigations (thick and thin blood films). Children presented with other causes of fever and those below two or above fifteen years of age were excluded from the study.

Sampling:

150 children attended KCEH during the study period were selected from the study population by convenience sampling.

Data collection:

Data were collected by a pre-coded, pre-tested questionnaire and the respondents were the mothers of children enrolled in the study. The clinical examination findings which include temperature measurement were taken and recorded. Laboratory investigations and disease progress were registered and the type of malaria was recorded.

Laboratory techniques:

Three mls of venous blood were taken from each patient. The serum was separated, freezed and kept under temperature of -20 to -70 degrees C. MSP-1 and MSP-2 anti bodies were detected by using indirect ELISA as follows:

1. The wells of 96 wells plate (immunol4 flat bottom plates from Dyntach laboratory INC, U.S.A.) were coated with 100 µl of recombinant protein fused to glutathione- S-transferase (GST).
2. 16 ng of MSP-1 (19), 50 ng of T9 /96 13/14 and 25 ng of GF 88 Full length of FC27 allele of MSP2 and 50 ng of GST in coating buffer is determined by check board titration.
3. The plates were incubated for three days at 4 degrees C.
4. The wells were washed five times with washing phosphate buffer saline (P.B.S.).

5. Unoccupied protein binding sites were blocked with 200 µl per well of blocking buffer (15 % W/V) skimmed milk powder in washing buffer for five hours in room temperature and again the wells were washed five times with washing buffer.
6. Sera diluted 1:500 in the blocking buffer (100 µl per well) were added to duplicate antigen- coated wells and incubate over night at 4 degrees C.
7. After five washings, the wells were incubated for three hours at room temperature with100 µl of substrate of conjugate – rabbit anti human IgG/HRP (DAKO) specific for gamma chain (1:5000).
8. The plates were washed again five times with washing buffer (PBS) and the wells were incubated for ten minutes with 100 ul of substrate buffer per well.
9. The reaction was stopped by adding 20 µl of 2 M H_2SO_4
10. The optical densities were measured at 492 nm.
11. Corrected optical density value for each serum sample was calculated by subtracting the mean optical density values of wells containing control (GST) protein alone from the mean optical density value obtained with each test antigen (GST).
12. Cut off values at which binding of antibodies from malaria exposed children were regarded as significantly above back ground calculated as the mean plus three standard deviations of the optical density reaching reading obtained with sera from Danish donors with no history of exposure to malaria (Used as control in this study).
13. It was calculated that significantly positive values in this study for MSP-1 would be 0.22 (mean +3 SD) and 0.32 for MSP-2. Consequently any sample of antibody value of 0.22 or above was considered positive for MSP-I and 0.32 or above as positive for MSP-2.

Data analysis:
Data were analyzed by the computer using SPSS soft ware.

Ethical concern:
Written consents were taken from the parents of the study population. Ethical clearance was also obtained from the health authorities.

Results:
Table (1) Seropositivity to MSP-1 and MSP-2 in Sudanese children

Type	Positive	Negative	Total
AntiMSP-1	69(46%)	81(54%)	(100%)
AntiMSP-1	63(42%)	87(58%)	(100%)

Discussion:

Almost half (46%) of the patients showed positive reaction to MSP-1 antigen compared to (54%) seronegative. This finding was lower than what had been reported from Senegalese children[15]. Iqbal investigated Pakistani children in Punjab and reported that children developed isolate specific antibodies during convalescence from an acute attack of P. falciparum[16]. It may well be due to a high degree of antigenic diversity of the parasite strain. Difference in endemicity plays a role in the different results[17].

Iqbal also reported high seropositivity (prevalence) in sera from African donors coming from Gambia, Madagascar and Liberia, and the findings of this study correspond to the results from Gambian children[17].

Considering the seropositivity to MSP-2 antigen in this study, 42% of the population studied was seropositive. This result is in line with data from Gambia which reported seropositivity to MSP-2 antigen between 47% and 88 %18. Genetic factors may be a cause, and higher seropositivity was associated with HLA class II alleles19. On the other hand, this finding is lower than that reported by Muller in Gambian children (50%)[10].

Table (2): Correlation between Anti-MSP1 and age in Sudanese children

	Age			
MSP-1 antibody	2-6 years	7-11 years	12-15 years	Total
Positive	20 (39.2%)	31(47.7%)	18(52.9%)	69(46%)
Negative	31(60.8%)	34(52.3%)	16(47.1%)	81(54%)
Total	51(100%)	65(100%)	34(100%)	150(100%)

X 2 = 1.67941, P= 0.43184, Not sig.

Table (3): Correlation between Anti-MSP2 and age in Sudanese children

	Age			
MSP-2 antibody	2-6 years	7-11 years	12-15 years	Total
Positive	22 (43.1%)	23 (35.4%)	18 (52.9%)	63 (42%)
Negative	29 (56.9%)	42 (64.6%)	16 (47.1%)	87 (58%)
Total	51 (100%)	65 (100%)	34 (100%)	150 (100%)

X^2 = 11.98996, P= 0.6219, Not sig.

Only 39.4% of the children aged 2 - 6 years were seropositve to MSP-1 compared to 47.7% of the patients 7 – 11 years old and 52.9% in 12 – 15 years age group. There was an increase in seropositivity with age but the findings were not statistically significant in contrast to Muller findings in Gambian children[10].

For MSP-2 seropositivity was found in 43% of the 2 – 6 years old children, 35.4% of 7 – 11 years old, and 52.9% of the 12 – 15 years old children. This increased seropositivity with age is consistent with Alyaman findings that highest seropositivity was found at 15 years of age. Immunity (seropositivity) is very slow to develop, and at times requiring 10 – 20 years. The slow build up of seropositivity with age and prolonged exposure was related to the conserved region of MSP-2 (Block 17– with less than 5% variation in different colones)[20].

Dizegel and his group conducted a longitudinal study in Gambia to assess malaria morbidity and antibody prevalence and concluded that seropositivty rates increased with age to a maximum of 77% for IgM, and 95% for IgG (MSP-1 and MSP-2) in adults[19].

Conclusion:-

MSP-1 antibodies were detected in 46% of Sudanese children compared to 42% who were seropositive to MSP-2. Co-existence of the antibodies to the two antigens was observed in 26.6% of the children compared to 43% who were seronegative to both antigens.

The antibody was age dependant, and findings were similar to what had been reported in some African countries. The study suggests the importance of further research in this area.

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