ANTI-MSP-1_{19} IGM ANTIBODIES IN CHILDREN EXPOSED TO *P. FALCIPARUM* IN IGBO-ORA SOUTH WESTERN NIGERIA*

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Abstract

Malaria remains a major parasitic disease in Africa, with children being the most affected. There is therefore an urgent need for the development of new effective measures, including vaccines. *Plasmodium falciparum* merozoite surface protein-1_{19} (MSP-1_{19}) is a prime candidate for a blood-stage malaria vaccine. The presence of IgM against *Plasmodium falciparum* has been linked to protection from malaria. This study intends to provide insight into the role of anti-MSP-1_{19} IgM in malaria immunity in children.

Blood was collected from 194 children aged 10 days to 15 years in the dry and rainy seasons of 1999. Parasite densities were determined by microscopy. Enzyme linked immunosorbent assay (ELISA), was used to determine the total IgM. The anti-MSP-1_{19} IgM antibody increased with age in both the dry and the rainy seasons (r_s=0.491 and r_s=0.571 respectively). There was also a positive correlation between anti-MSP-1_{19} IgM antibody and age for *P.falciparum* positive or negative individuals in the dry and the rainy seasons respectively.

The results from this study imply that Anti-MSP-1_{19} IgM may play a protective role in the anti-malarial immunity of children.

Key Words: Malaria; parasitemia; IgM; *Plasmodium falciparum*; merozoite surface protein-1_{19}; Age.

1. Introduction

MSP-1_{19} has been shown to be a target of protective immunity and is a leading vaccine candidate currently under development. Some MSP-1_{19} specific antibodies that inhibit merozoite invasion also inhibit the secondary processing of MSP-1 (Blackman et al., 1994), and this is proposed to be the basis of their protective mechanism. Processing inhibitory antibodies have also been reported to occur in individuals naturally exposed to malaria (Nwuba et al., 2002). MSP-1 is synthesized by the intracellular schizonts as a high molecular-mass precursor (185-220 kDa) and undergoes two steps of proteolytic processing during the maturation of merozoite (Holder and Freeman, 1984; Blackman *et al.*, 1990; Blackman *et al.*, 1991a; Blackman *et al.*, 1991b; Blackman et al., 1993; Blackman et al., 1994 and Blackman and Holder, 1992). Just prior to or at the point of merozoite release from the mature schizonts, the MSP-1 precursor is cleaved into four fragments referred to as MSP-1_{83}, MSP-1_{30}, MSP-1_{38} and MSP-1_{42}, which exist as a non-covalently associated complex on the free merozoite surface (Holder and Freeman, 1984; Holder, 1988). At some point following merozoite release, a second processing event takes place, in which the C-terminal MSP-1_{42} is cleaved into two fragments of approximately 33 and 19 kDa (referred to as MSP-1_{33} and MSP-1_{19}) (Blackman and Holder, 1992).

Animals that are vaccinated with the protein develop high titres of anti MSP-1 antibodies and the level of the antibodies corresponds to the level of protection. Partial protection against *P. falciparum* challenge was achieved by immunization with recombinant MSP-1_{19} protein while complete protection was observed with protein purified from the parasite (Kumar et al., 2000; Holder 1993). In healthy individuals and adults naturally exposed to malaria there appears to be epitope specific naturally acquired MSP-1_{19} immune response boosting after vaccination (Lee *et al.*, 2002).

Anti-MSP-1_{19} IgM plays a role in immune protection from malaria, parasite specific IgM was detected in 14% of cord blood samples, and these IgM antibodies reacted to a range of asexual stage antigens, with each newborn having its own unique pattern of IgM reactivity. Maternal placental malaria and anaemia was associated with the production of *P. falciparum* IgM by the foetus (King *et al.*, 2002). It was also

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reported that 6% of newborns had cord blood anti-MSP-1_{19} IgM antibodies, IgM or IgE antibodies to malaria antigens constitutes evidence of in utero sensitisation, the cord blood IgM was restricted to the MSP-1_{19} (Xi et al., 2003). There was no significant correlation of anti-MSP-1_{19} IgM in maternal samples with cord blood of the children.

It has been reported that multiple anti-MSP-1_{19} IgM responses are associated with infants multiple infections and that these responses were protective; and when the levels decreased another infection quickly followed (Branch et al., 1998). The same authors reported that the first IgG and IgM peaks coincided 92% of the time, while overall the IgM accompanied the IgG production 83% of the time (Branch et al., 1998). It has been shown that anti-MSP-1_{19} IgM predominated over anti-MSP-1_{19} IgG in a particular town in Senegal, and this IgM production could result from reinfection with different parasite strains or with parasite expressing allelic variations (Nguer et al., 1997). In this study we intend to determine the role of anti-MSP-1_{19} IgM in children in a malaria endemic area.

2. Materials and Methods

MSP-1_{19} Antigen

Wild type MSP-1_{19} recombinant proteins were obtained from Dr. Tony Holder, National institute for Medical Research (NIMR), Mill Hill, London, England.

Study Area

Igbo- Ora and Idere towns in Ibarapa local government area of Oyo state in south-western Nigeria were chosen as the study site. Igbo-Ora is in the savannah region, which has numerous small streams. *Anopheles gambiae* and A. *funestus* are the mosquito species found in this area (Lawrence, 1965). The climate consists of a warm dry season (November to March) and a cooler rainy season (April to October). The main occupation of the men is farming and hunting while the women are peasant farmers and retail traders (Achidi et al., 1996).

Study Design

The study protocol was reviewed and approved by the Joint Ethical Committee of the College of Medicine and the University College Hospital, Ibadan. The subjects of the study included infants and children from 10 days to 15 years. Individuals were enrolled in the dry and rainy seasons respectively, criteria for inclusion into the survey included the age, sex, length of time spent in the study site and informed consent.

Blood Collection

Blood (1-2 ml) was collected by venipuncture from the arm by qualified medical doctors. The blood was then stored in sample tubes with 0.12M trisodium citrate in them and properly labelled, the tubes containing the blood was then stored in ice, and then transported to the Cellular Parasitology Laboratory in Ibadan within 3 hours, where it was the centrifuged at 8,000 rpm for 2 minutes in a Biofuge table top centrifuge (Hereaus instruments, Kendro Lab product GmbH, Lagenselbold, Germany). The plasma obtained from it was stored at –80° C (Forma Scientific, Marietta, Ohio, USA).

Parasitology

Blood was spotted on the slide and thick and thin films were prepared. The slides were labelled, allowed to dry, stored in a slide rack and transported to Ibadan. The thick film was then stained in 10% Giemsa solution (buffered distilled water pH 7.2) in a Wheaton staining jar for 20 minutes and then allowed to dry. The thin film was first fixed in methanol before being immersed in 10% Giemsa solution for 20 minutes. The parasites were counted with a microscope (Leitz Laborux-11, Germany) using the thick film on the basis of number of parasites per 200 white Blood Cells; this was converted to the number of parasites per µl of blood (WHO 1985).

Determination of anti-MSP-1_{19} IgM by ELISA

MSP-1_{19} specific IgM of 194 samples, 101 in the dry season and 93 in the rainy season was determined by ELISA. Flat bottom polyvinyl chloride plates (Corning Incorporated-Life Sciences, MA, USA) were coated with 50 µl of MSP-1_{19} (0.5ug of MSP-1_{19}/ml of sodium carbonate buffer) and incubated overnight at 4° C. The antigen was poured off and the Plates were then blocked with 150 µl of 0.5% Boiled Casein for 1 hour at 37°C. The plates were washed three times with PBS-Tween 20. Sera serially diluted from 1:20-1:2560 in boiled casein were added to the plates that were then incubated for 1 hour at 37°C in an incubator (Forma Scientific, Marietta, Ohio, USA). The plates were washed three times with PBS-Tween 20 and 50 µl of 1:1000 dilution of Goat anti Human IgM (µ-chain specific) peroxidase conjugate in boiled casein was added to the plates which were then incubated for 1hour at 37°C in the incubator (Forma Scientific, Marietta, Ohio, USA). The plates were then finally washed three times with PBS-Tween 20. 50 µl of ABTS/H_2O_2 was added to the plates and the colour allowed to develop at 37 °C for 30 minutes in the incubator (Forma Scientific, Marietta, Ohio, USA), the absorbance was read at 650nm with a microplate reader (Molecular Devices, Menlo Park, CA, USA) without stopping the reaction.

Statistical Analysis

The antibody titres were log transformed and the results analyzed using correlation, logistic regression, ANOVA and students t test. The levels of significance were estimated at $P< 0.05$ for logistic regression, ANOVA and students t test, while $P< 0.01$ for correlation. The log reciprocal antibody titres were expressed at log base 10. The software packages used were Microsoft EXCEL and SPSS.

3. Results

The study showed that in the dry season 46% of the children where *P.falciparum* positive while at the end of the rainy season 58% were *P.falciparum* positive (Table 1). The IgM titres were determined in both dry and rainy seasons of 1999. There was a significant positive correlation between age and anti MSP-1_{19} IgM antibody titre (rs=0.491) in the dry season (Fig. 1). At the end of the rainy season there was a significant positive correlation between age and anti MSP-1_{19} IgM IgM titre (rs=0.571) (Fig. 2). Anti MSP-1_{19} IgM titre for the dry season samples and at the end of the rainy season samples were compared by the student t test and the result showed that there was no significant difference in the anti MSP-1_{19} IgM between the two groups. The influence of anti-MSP-1_{19} IgM, age and sex on malaria was analysed using regression analysis. The result of the analysis of variance between the three factors and malaria outcome showed that the predictors had no significant effect on the outcome. The actual regression then showed that none of the predictors had an effect in predicting *P.falciparum* positive children in the dry season. However, at the end of the rainy season, analysis of variance between the three factors and malaria outcome showed that the predictors had no significant effect together, but age on its own was significant and could be used to predict individuals with *P. falciparum* at the end of the rainy season ($p<0.05$). There was positive correlation between anti-MSP-1_{19} IgM and age for *P.falciparum* positive samples in both the dry season (rs=0.386)(Fig. 3) and at the end of the rainy season (rs= 0.686)(Fig. 5).There was also a positive correlation between anti-MSP-1_{19} IgM and age for *P.falciparum* negative samples in both the dry season (rs=0.417)(Fig. 4) and at the end of the rainy season (rs= 0.557)(Fig. 6). There was no significant difference in the anti-MSP-1_{19} IgM titres for *P.falciparum* positive or negative individuals in the dry season and at the end of the rainy season. There was a negative correlation between parasitemia and anti-MSP-1_{19} IgM titre.

4. Discussion

Several studies have shown that immunoglobulin purified from the blood of immune adults from endemic regions can passively transfer protection against *P. falciparum* (Edozein et al., 1962; Mcgregor 1964). Malaria vaccines currently on field trials have not been successful though they elicit immune response (Plebanski, 2002). In this study MSP-1_{19} an asexual stage malaria vaccine candidate was the antigen used for immunoepidemiological analysis. Antigens are favoured as candidates if they are accessible to the immune system, induce protective immune responses in animal models, and either lack antigenic diversity or have at least limited diversity (Richie and Saul, 2002). The anti-MSP-1_{19} IgM titre increased with age in both seasons (Figs. 1 & 2) showing that individuals had increased anti-MSP-1_{19} IgM titres as they grew older and had more frequent contact with the parasite. It has been shown that IgM levels in children increased with age and that the increment was marked after the age of seven (Aribot et al., 1996). These levels were gradually and consistently increased during the highest transmission season. A previous study in Igbo-Ora showed that mean IgM levels against the antigen Pf155/RESA and Ag332 or the circumsporozoite protein (CSP) increased throughout the first year of life (Achidi et al.,1995). There was no significant difference in the anti MSP-1_{19} IgM titre for the dry season and at the end of the rainy season. A previous publication have reported that MSP-1_{19} specific IgM was found in samples collected in areas with perennial and intense transmission and also in areas during low transmission season. There was no significant difference in anti-MSP-1_{19} IgM titres for *P.falciparum* positive or negative individuals in the dry season and at the end of the rainy season. The results of this study showed that there was a positive correlation between anti-MSP-1_{19} IgM titres and age for *P.falciparum* positive or negative individuals in the dry season and rainy seasons (Nguer et al., 1997) (Figs. 3-6). This might imply that age plays a strong role in determining the anti-MSP-1_{19} titre, but it should be noted that individuals who are *P.falciparum* negative might have previously had *P.falciparum* infection. Regression analysis showed that in the dry season anti-MSP-1_{19} IgM titre, age and sex had no effect on malaria out come. At the end of the rainy season only age had an effect on malaria outcome ($p<0.05$). This might mean that anti-MSP-1_{19} IgM and other antibody isotype increase as an individual grew older in the rainy season. There was a negative correlation

Table 1: Prevalence of *P.falciparum* in the dry and rainy seasons. This table shows the number and percentages of individuals that were *P. falciparum* positive or negative during the dry and rainy seasons.

Season	***P. falciparum*** negative N (%)	***P.falciparum*** positive N (%)
Dry season N=101	54 (54)	46 (46)
Rainy season N=93	39 (42)	54 (58)

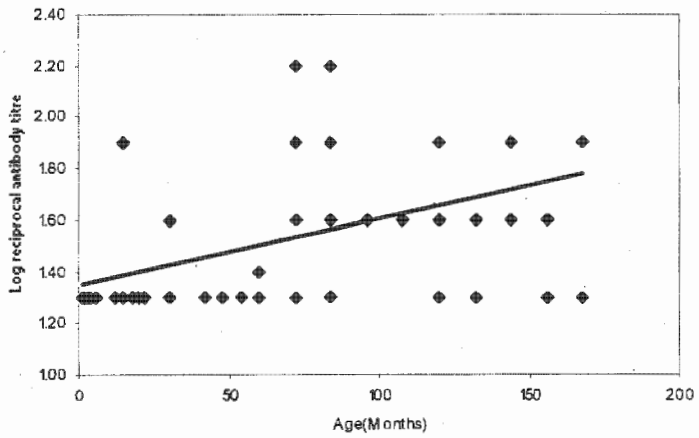


Fig. 1: Correlation between anti-MSP-1_{19} IgM and age in the dry season. Scatter diagram showing the relationship between age and anti-MSP-1_{19} IgM antibody titre for children in the dry season. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.

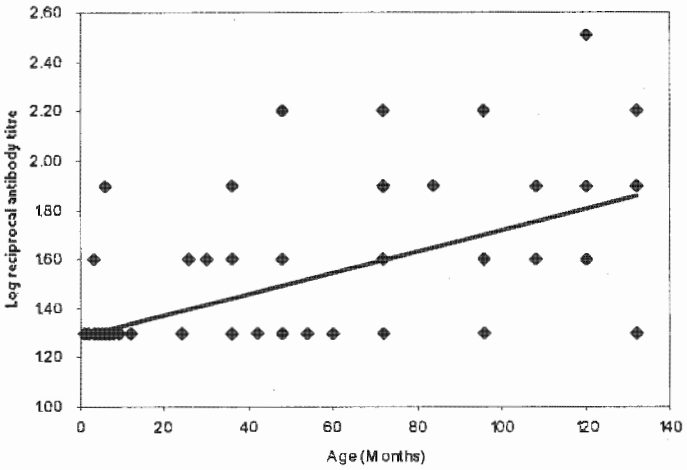


Fig. 2: Correlation between anti MSP-1_{19} IgM and age at the end of the rainy season. Scatter diagram showing the relationship between age and anti-MSP-1_{19} IgM antibody titre for children at the end of the rainy season. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.

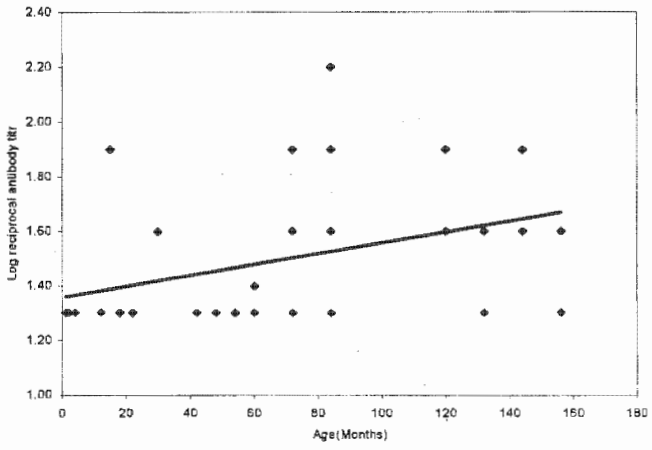


Fig. 3: Correlation between anti-MSP-1_{19} IgM and age for *P. falciparum* positive samples in the dry season. Scatter diagram showing the relationship between age and log anti-MSP-1_{19} IgM antibody titre for children whose giemsa stained blood smears were *P. falciparum* positive. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.

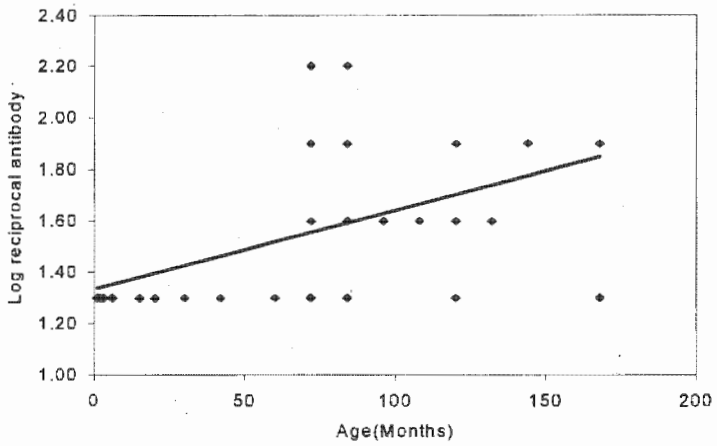
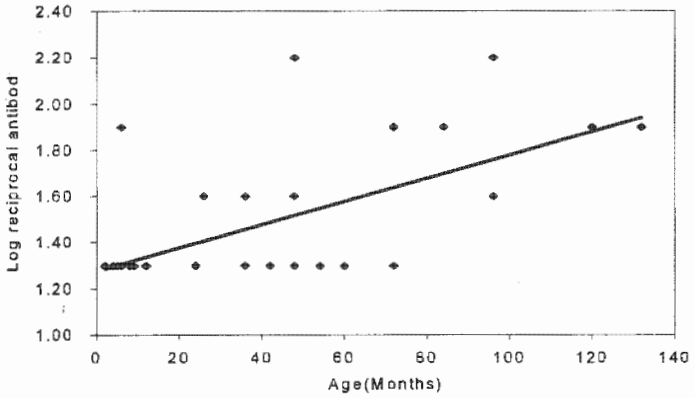
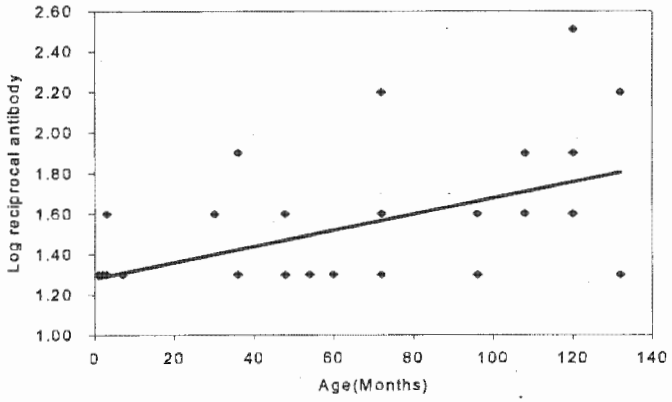


Fig. 4: Correlation between anti-MSP-1_{19} IgM and age for *P. falciparum* negative samples in the dry season. Scatter diagram showing the relationship between age and log anti-MSP-1_{19} IgM antibody titre for children whose giemsa stained blood smears were *P. falciparum* negative. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.



Correlation between anti-MSP-1_{19} IgM and age for *P. falciparum* positive samples in the rainy season. Scatter diagram g the relationship between age and log anti-MSP-1_{19} IgM antibody titre for children whose giemsa stained blood smears were *P. falciparum* positive. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.



Correlation between anti-MSP-1_{19} IgM and age for *P. falciparum* negative samples in the rainy season. Scatter diagram g the relationship between age and log anti-MSP-1_{19} IgM antibody titre for children whose giemsa stained blood smears were *P. falciparum* negative. Antibody titre is expressed as log reciprocal antibody titre, while age is in months.

between parasite density and anti-MSP-1 IgM titre; showing that IgM could play a role in immunity to malaria corroborating another study which have previously reported that Anti-MSP-1_{19} IgM helped in parasite clearance and was protective (Branch *et al.*, 1998). Results from this study suggest that the children living in Igbo-Ora have some degree immunity against malaria and this increase as they grow older. This immunity might be as a result of the high levels of anti-MSP-1_{19} IgM.

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