Effect of Seasonal Malaria Chemoprevention (SMC) with Sulfadoxine-Pyrimethamine (SP) and Amodiaquine (AQ) on the Acquisition of anti-AMA1 and anti-MSP1_42 Antibodies among Children Under 10 Years Living in the Southern part of Senegal (Velingara)

Khadime Sylla1*, Roger Clément Kouly Tine1, Doudou Sow1, Magatte NDiaye1, Aissatou Sarr1, Marie Louise Tshibola Mbuyi2, Ibrahima Diouf1, Jean Louis Abdourahim Ndiaye2, Daouda NDiaye1, Oumar Gaye1 and Babacar Faye1

1Service of Parasitology-Mycology, Pharmacy and Dentistry, University Cheikh Anta Diop, Dakar, Senegal
2Department of Parasitology-Mycology, University of Health Sciences, Libreville, Gabon

Abstract

Background: In developing countries, malaria is still a leading cause of morbidity and mortality and children are the most affected individuals. In order to strengthen malaria control, new intervention such as Seasonal Malaria Chemoprevention (SMC) has been developed. This strategy is very effective in preventing malaria clinical episodes but its effect on children’s immunity is not well documented. This study aimed to evaluate the effects of SMC on the acquisition of anti-AMA1 and anti-MSP1_42 antibodies among children fewer than 10 years living in the southern part of Senegal (Velingara).

Patients and methods: The study was nested in a cluster randomized trial assessing the impact of SMC with a single dose of Sulfadoxine-Pyrimethamine (SP) and 3 doses of Amodiaquine (AQ). Two cross-sectional surveys were carried out (October 2010) and (September 2011) to assess the effect of SMC on children’s immunity. Thick and thin blood smears were performed to assess malaria parasitaemia prevalence. Blood was collected on filter paper for serological measurement by ELISA to measure IgG anti-MSP1_42 and anti-AMA1. Logistic regression analysis was performed to assess factors associated with the production of antibodies.

Results: A total number of 1611 children under 10 years old were included in two surveys (866 children in 2010 and 745 children in 2011). Malaria prevalence was 10.39% at baseline (2010) and 5.03% one year after intervention (2011). The seroprevalence of anti-MSP1_42 anti-AMA1 antibodies was higher in 2010 compared to 2011 providing a significant reduction of IgG production at 11.4 AU (95%CI [8.3-14.4]) for MSP1_42 and 7.2 AU (95%CI [4.5-9.9]) for AMA1. Seroprevalence increased with age and Plasmodium falciparum carriage while it decreased according to the area and study period.

Conclusion: SMC is an effective strategy for malaria prevention in children under 10 years. The strategy can as well induce a decrease of IgG anti-AMA1 and anti-MSP1_42 which are protective against malaria. Consequently, this strategy needs to be renewed every year in areas where malaria is highly seasonal to avoid a resurgence of malaria, while promoting the use of other antimalarial interventions.

Keywords: Malaria; Plasmodium falciparum; SMC; Children; Immunity; Senegal

Introduction

Malaria is still a leading cause of morbidity and mortality despite all the efforts made to control the disease. Over 80% of malaria cases and 90% of malaria deaths occur in Africa and mainly in children [1]. In order to strengthen the fight against malaria in children, a new preventive strategy was recently developed: intermittent preventive treatment currently called Seasonal Malaria Chemoprevention (SMC). This strategy is defined as the administration of therapeutics doses of antimalarial drugs at monthly interval during malaria transmission period in areas where malaria is endemic. Several studies in Africa have shown that this intervention, cost-effective, safe, and feasible for the prevention of malaria among children in areas with highly seasonal malaria transmission. In Senegal, Cisse et al. showed 86% of reduction of malaria incidence among children who received seasonal intermittent preventive treatment [2]. A study in Mali showed a 67.5% efficacy of IPT of 67.5% against clinical malaria episodes [3]. In Ghana, Kwedu et al. found 69% efficacy of in terms of reducing malaria incidence [4]. In Tanzania, Schellenberg et al. showed a protective effect of 36% of intermittent preventive treatment in children [5]. WHO adopted in March 2012, Seasonal Malaria Chemoprevention (SMC) previously referred to as intermittent preventive treatment for malaria prevention strategy in children living in the Sahel sub in regions of Africa [6,7].

This strategy is certainly effective, but it may induce an effect on immunity by reducing production of malaria antibodies such as anti-MSP1_42 (Merozoite Surface Protein) and anti-AMA1 (Apical Membrane Protein) which are associated with protection against malaria. Immunization with these antigens provides protection against
malaria. Several studies have shown that SMC decreased the production of protective antibodies against malaria [8,9].

This study was conducted to evaluate the effects of SMC with Sulfadoxine-Pyrimethamine and (SP) and Amodiaquine (AQ) on the acquisition of anti-AMA1 and anti-MSP1_42 antibodies in children under 10 years living in the southern part of Senegal (Velingara).

Methodology

Study area

This study was carried out in Velingara health district located in the South-eastern part of Senegal, 500 km from the capital city of Dakar. In this district the study was conducted in Bonconto health post headed by a nurse and has 8 functional health huts staffed with community health workers, serving a total population of 10,016 inhabitants. Malaria transmission is seasonal during rainy season (from July to November) with a peak between October to November. Plasmodium falciparum is the most predominant parasite species. Malaria control strategies implemented by the National Malaria Control Program (NMCP) were represented by the case management of uncomplicated malaria cases using RDTs and ACTs; intermittent preventive treatment in pregnant women; universal coverage of long lasting insecticide treated net and indoor residual spraying.

Study design

The study was nested in a cluster randomized trial assessing the impact of SMC with a single dose of Sulfadoxine-Pyrimethamine (SP) and 3 doses of Amodiaquine (AQ) on the incidence of malaria clinical episodes, malaria parasitaemia and anaemia prevalence at the end of transmission season. Children in intervention area were assigned to receive SMC plus community case management of malaria (CCm) while in the control area children had only access to CCm. Details for the cluster randomized trial procedures are described previously [10]. For the immunological assessment, a controlled before and after study was done to assess factors associated with malaria parasiteamia prevalence at the end of transmission season. Children in intervention and control areas, (ii) seroprevalence of anti-AMA1 from baseline to end line both in intervention and control areas, (iii) malaria parasitaemia prevalence at cross sectional surveys.

Data collection method

A questionnaire was administered to collect individual’s socio-demographic data (age, gender, weight, height, area of residence), clinical information, and access to antimalarial interventions such as bed net. Anthropometric data were collected as previously described [11]. Blood samples were collected using finger prick blood for malaria diagnostic, haemoglobin and antibody measurement. Haemoglobin level was measured using Hemo-Cue machine (Hemocue® Hb 301). Anemia was defined as Hb concentration below 11 g/dl.

Evaluation of anti-Plasmodium falciparum IgG antibodies by ELISA

Three drops of blood were collected onto Whatman 3MM filter paper, which was sealed and stored dry with desiccant at room temperature. Reconstituted sera were obtained from filter paper bloods spots described elsewhere [12,13]. Sera were tested for anti-MSP1_42 IgG antibodies and anti-AMA1 IgG antibodies by indirect ELISA. Apical membrane antigen (AMA1) was from the Pichia pastoris expressed ectodomain of Plasmodium falciparum FVO strain comprised amino acids 25–545 [14]. MSP1_42 protein was from the C-terminal MSP1_42 amino acid sequence of the Uganda-Palo Alto (FUP) P. falciparum isolate expressed in Escherichia coli (Ec) system [15]. Samples were also tested on freeze thawed P. falciparum Schizont Extract (concentration of 1 x 10^7/ml), which was coated onto ELISA plates at 1/500.

Briefly, 96 well ELISA plates were coated with 100 µl/well of 0.1 µl/well of MSP1 and 0.026 µl/well of AMA1 in coating buffer (1.59 g NaH2PO4, 2.93 g NaHCO3, 1 liter distilled water, pH 9.4). The plates were incubated overnight at 4°C. After incubation, plates were washed at three times using PBS (5.7 g NaH2PO4, 16.7 g Na2HPO4, 85 g NaCl in 1 liter distilled water) plus 0.05% Tween 20 (PBS/T) and blocked with 1% (w/v) skimmed milk power in PBS/T for one hour at 37°C. Eluates were removed from 4°C just before use. Following three more washes, eluates were diluted at a ration 1/100 in PBS/T and added 100 µl in duplicate in a well plate.

For each plate three types of control were used: deep well without serum but with a second antibody to measure the non-specific binding, pool of sera from patients with Plasmodium falciparum malaria (positive control) and pool of sera from non-infected subjects (negative control) from Copenhagen. Plates were incubated one hour at 37°C. After three more washes 100 µl of horseradish peroxidase-conjugated rabbit anti-human IgG (SouthernBiotec ) (1/5000 in PBS/T) was added to all wells.

After incubation for one hour at 37°C, plates were developed with TMB/E (Upstate®, Chemicon® et Linco®, Millipore) as substrate for 30 minutes at room temperature and the reaction was stopped by the addition of 50 µl/well of 2M H2SO4. Optical density was read at 450 nm against a 620 nm with an ELISA TECAN SUNRISE reader.

Statistical Methods

Sample size calculation

For each cross sectional survey the total number of children to examine was calculated at 800, based on a prevalence of malaria parasitaemia at 20% in the study area (Senegal MIS 2009) a confidence level at 95% with a precision of 5%, power level at 90% and assuming a percentage of 20% of withdrawal.

Data management and data analysis

Data were entered in Excel software and analysis was performed using Stata software version IC 12.1 software. For serological assessment, the optical density was obtained by subtracting the average OD of duplicate wells from that of the corresponding blank wells. Values were converted into arbitrary units (AUs), as previously described [16]. Quantitative variables were described in terms of means, standard deviation. Inter group comparisons were done using ANOVA test or Student t test where appropriate; otherwise non parametric tests such as Man withnney or Kruskall Wallis) were used. For descriptive data, percentage was used to each outcome. Antibodies seroprevalence was calculated and expressed by percentage with their 95% confidence intervals. Proportions were compared using chi-square test or the Fisher exact test (univariate analysis). A stepwise logistic regression analysis was done to assess factors associated with Pf antibodies carriage. Significance level of the different tests was set at 5%.

Ethical Considerations

Informed consent was required prior the participation in the
study. Ethical approval was obtained from the Senegalese National Ethical Committee (Conseil National d’Ethique et de Recherche en Santé). Approval number 027/MSP/DS/CNRS, 18/03/2010. The study was registered at the Pan African Clinical Trial Registry: registration number: PACTR201305000551876.

Results

Baseline characteristics of study population

A total of 1611 children under 10 years were included in this study (866 children in 2010 and 745 children in 2011); 450 children in 2010 and 470 children in 2011 received SMC while 416 children in 2010 and 275 children in 2011 were included in control area.

The mean age of study participants was 4.5 ± 2.7 years and 4.02 ± 2.3 years respectively in 2010 and in 2011. Study population was mainly represented by children over 5 years old (60.64%) in 2010 and 470 children in 2011. The sex ratio was 0.95 in 2010 and 1.01 in 2011.

The mean hemoglobin level was 8.5 ± 3.4 g/dl and 9.3 ± 1.8 g/dl respectively in 2010 and 2011. The prevalence of anemia (Hb <11 g/dl) represented by children over 5 years old (60.64%) in 2010 and children 2.3 years respectively in 2010 and 2011.

Prevalence of anemia (Hb <11 g/dl) was registered at the Pan African Clinical Trial Registry: registration number: PACTR201305000551876.

Anti-plasmodium IgG responses

In 2010 the level of IgG anti-MSP1,42 and IgG anti-AMA1 was respectively 23.2 AU and 17.2 AU while in 2011 it was 11.8 AU and 9.9 AU for both antibodies.

Comparing 2010 and 2011, these results showed a decrease of IgG anti-MSP1,42 and IgG anti-AMA1. The difference was significative (p<10-3). The mean difference of IgG anti-MSP1,42 and IgG anti-AMA1 between 2010 and 2011 was respectively 11.4 AU (95% CI[8.3-14.4]) and 7.2 AU (95% CI[4.5-9.9]). At baseline (2010), in 2010, the level of IgG anti-MSP1,42 and anti-AMA1 was respectively 25.2 AU and 22.1 AU in SMC area while it was respectively 23.2 AU and 17.2 AU while in 2011 it was 11.8 AU and 9.9 AU in SMC area.

In control area a decrease of IgG anti-MSP1,42 was noted between 2010 and 2011. The mean difference was 7.2 AU (p<10-3). An increase of IgG anti-AMA1 was noted between 2010 and 2011. The mean difference was -1.3 AU (p=0.38).

In SMC area, a significant decrease of IgG anti-AMA1, and IgG anti-MSP1,42 was not observed between 2010 and 2011 (p<10-3). Mean differences observed were 12.713 AU and 0.4 AU respectively both antibodies.

Overall, the seroprevalence rate of anti-MSP1,42 and anti-AMA1 antibodies was higher in 2010 (at baseline) compared to 2011 (a year after intervention). Anti-MSP1,42 and anti-AMA1 antibody prevalence was respectively 53.12% and 46.3% in 2010. While in 2011 the seroprevalence of both antibodies was 20.03% and 19.8%.

Regarding the area (SMC area / Control area) the seroprevalence of anti-MSP1,42 and anti-AMA1 antibody was higher in control zone. In 2010, the seroprevalence of both antibodies was respectively 62.76% and 54.81% in the intervention area while it was respectively 44.22% and 38.44% in the control area. The difference was significative (p<10-3). In 2011, a year after SMC implementation, the seroprevalence rate of anti-MSP1,42 and AMA1 antibodies was 24.36% and 22.18% respectively in control area. However in the SMC zone, the seroprevalence of both antibodies was 17%.

In SMC zone, the seroprevalence of anti-MSP1,42 antibody decreased by 2.5 folds between 2010 and 2011. For anti-AMA1 antibodies the seroprevalence rate decreased by 0.5 folds (Figure 2).
Table 4: Level of IgG anti-MSP1_42 and anti-AMA1 in SMC area and control area depending to the year.

(aOR=1.17; 95% CI (0.81 - 1.68), p value=0.38). The seroprevalence of 39.7% (aOR=1.07; 95% CI (0.75 - 1.54), p value=0.68) and 39% antibodies was higher in children over 5 years old with respectively the area (SMC + / SMC-).

Multivariate analysis showed that seroprevalence of anti-AMA1 and anti-MSP1_42 antibodies increases with age and Plasmodium falciparum carriage. Seroprevalence of anti-MSP1_42 and anti-AMA1 antibodies was higher in children over 5 years old with respectively 39.7% (aOR=1.07; 95% CI (0.75 - 1.54), p value=0.68) and 39% (aOR=1.17; 95% CI (0.81 - 1.68), p value=0.38). The seroprevalence of anti-MSP1 and anti-AMA1 antibodies was more important in children with malaria infection compared to subject without malaria infection. In children with malaria infection, seroprevalence of anti-MSP1_42 and anti-AMA1 was respectively 40.94% (aOR=1.13; 95% CI (0.77 - 1.63); p value=0.22) and 37.01% (aOR=1.16; 95% CI (0.81-1.69); p value=0.44). However, a decrease of anti-AMA1 and anti-MSP1 antibodies was observed according to the area and the study period. The seroprevalence of antibody was lower in SMC area compared to control area. Prevalence of anti-MSP1 and anti-AMA1 was respectively 30.63% (aOR=0.53; 95% CI (0.43 - 0.66), p value<10^(-3)) and 27.68% (aOR =0.56; 95% CI (0.45 -0.71), p value<10^(-3)) in SMC area. These result showed a protective effect of seasonal malaria chemoprevention to 47% for MSP1_42 and 44% for AMA_1. In control zone, the seroprevalence of both antibodies was 47.47% and 41.82%.

The seroprevalence of both antibodies was higher in 2010 compared to 2011. In 2011, the prevalence of anti-MSP1_42 antibodies was 20.03% (aOR=0.23; 95% CI (0.18 - 0.28), p value<10^(-3)). For anti-AMA1 antibody, it was 19.08% (OR=0.29; 95% CI (0.24 - 0.37), p value<10^(-3)). These results showed a protective effect of IPT around anemia and nutritional status.

Discussion

In developing country malaria is still a leading cause of morbidity and mortality in children. This situation represents a major public health problem. To reduce malaria burden in children, WHO recommend in March 2012 Seasonal Malaria Chemoprevention (SMC) as a malaria preventive strategy in children [6,7]. This strategy has been shown to be effective, cost-effective, safe, and feasible for the prevention of in endemic areas. However, the influence of SMC on acquired immunity

Table 2: Level of IgG anti-MSP1_42 and anti-AMA1 in 2010 and 2011.

Table 3: Level of IgG anti-MSP1_42 and anti-AMA1 in 2010 and 2011 depending on the area (SMC + / SMC-).

Table 4: Level of IgG anti-MSP1_42 and anti-AMA1 in SMC area and control area depending to the year.

Table 5: Multivariate adjusted analysis for the risk factors of anti-MSP1_42 antibodies.
In SMC was in line with results observed by Boulanger et al. when SMC area where seroprevalence of antibodies was lower. Similar results of malaria antibodies was more important in control area compared to 2010 and 2011. One year after SMC implementation, the seroprevalence of malaria antibodies decreased between 2010 and 2011 and it was higher in children who received SMC with SP plus AQ compared to those in control area. Overall, a year after SMC implementation a decrease of protective treatment in Senegalese children compared to control group [8]. In Gambia a decrease of malaria antibodies production was observed in children who received IPT with Pyrimethamine and Dapsone [19].

Our findings were similar with what was observed in Ghanaian children six months after intermittent preventive treatment with single dose of SP [20]. However, previous studies have shown no significant difference between IPT group and control group in terms of antibody production [21-23]. Overall, a year after SMC implementation a decrease of protective antibodies against malaria was noted. This situation increases the susceptibility of children to malaria infection. Indeed, a year after SMC implementation *Plasmodium falciparum* malaria, prevalence was higher in areas where children had access to SMC compared to control area. Similar findings concerning the rebound effect of SMC were described previously. In Ghana an increase of malaria incidence by 62% in children who had access to intermittent preventive treatment a year after intervention was observed [19].

Multivariate analysis, showed that seroprevalence of *anti-AMA1* and *anti-MSP1* antibodies is correlated with age, specifically in children over 5 years [29]. These results are in line with those found in Ghana that showed IgG levels increased with age, specifically in children over 5 years [29]. These results are in line with what was observed in the in northern part of Senegal with an increase of IgG according to the age [28]. Previous study conducted in Senegal showed that antibodies level increases with age, specifically in children over 5 years [29]. These results are in line with what was observed in the in northern part of Senegal with an increase of IgG according to the age [28].

A decrease of anti-MSP1 and anti-AMA1 antibodies was observed between 2010 and 2011 and it was higher in children who received SMC with SP plus AQ compared to those in control area. Overall, the seroprevalence of malaria antibodies decreased between 2010 and 2011. One year after SMC implementation, the seroprevalence of malaria antibodies was more important in control area compared to SMC area where seroprevalence of antibodies was lower. Similar results were found by other authors. The low prevalence of malaria antibodies in SMC was in line with results observed by Boulanger et al. when assessing the immunological consequences of intermittent preventive

<table>
<thead>
<tr>
<th>Table 6: Multivariate adjusted analysis for the risk factors of anti-AMA1 antibodies.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age group</strong></td>
</tr>
<tr>
<td>1 year (0-2 years)</td>
</tr>
<tr>
<td>2-5 years</td>
</tr>
<tr>
<td>5-10 years</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td><strong>Nutritional status</strong></td>
</tr>
<tr>
<td>Stunting</td>
</tr>
<tr>
<td>Underweight</td>
</tr>
<tr>
<td>Wasting</td>
</tr>
<tr>
<td><strong>Anemia</strong></td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td><strong>Malaria Pf</strong></td>
</tr>
<tr>
<td>No</td>
</tr>
<tr>
<td>Yes</td>
</tr>
<tr>
<td><strong>Area</strong></td>
</tr>
<tr>
<td>Control area</td>
</tr>
<tr>
<td>SMC area</td>
</tr>
<tr>
<td><strong>Study period</strong></td>
</tr>
<tr>
<td>2010</td>
</tr>
<tr>
<td>2011</td>
</tr>
</tbody>
</table>

Note: Seroprevalence of IgG antibody to AMA1 (Apical Membrane Protein) and MSP1 (Merozoite Surface Protein) in 2010 and 2011. Seroprevalence of both antibodies was more important in 2010 compared to 2011. Significant reduction of anti-MSP1 and anti-AMA1 antibody was observed after intervention in SMC area (p<10^-3). Black: anti-MSP1 antibodies. Grey: anti-AMA1 antibodies.

Figure 2: Seroprevalence of anti-MSP1 and anti-AMA1 antibodies in 2010 and 2011 in SMC and control area.
The seroprevalence of anti-MS1 and anti-AMA1 antibodies was lower in 2011 than in 2010. The low prevalence of malaria antibodies may be due by the decrease of malaria prevalence and the effect of seasonal malaria chemoprevention.

Conclusion

SMC is an effective strategy for malaria prevention in children under 10 years. The strategy can as well induce a decrease of IgG anti-AMA1 and anti-MS1, which are associated with protection against malaria. Consequently, this strategy needs to be renewed each year in areas where malaria is highly seasonal to avoid a resurgence of malaria, while promoting the use of other antimarial interventions.

Competing Interests

The authors declare that they have no competing interests.

References