Original article

Schistosoma haematobium and Plasmodium falciparum co-infection with protection against Plasmodium falciparum malaria in Nigerian children

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Abstract

Objective: Malaria remains the single leading killer of children in sub – Sahara Africa and Schistosomiasis is considered to be second to malaria in global importance. Co – infection of malaria and urinary schistosomiasis has been reported to exacerbate disease morbidity such as anaemia. In different part of the globe, the co – infection between malaria and schistosomiasis provides some protections on the infected persons. The protective effect of this co – infection elucidated immunologically using cytokines is lacking in our locality. **Methods**: Urine and blood samples obtained from the 160 volunteers were subjected to standard parasitological techniques for diagnosis of urinary schistosomiasis and malaria respectively. Blood samples collected from these volunteers comprising 80 children with schistosomiasis and malaria and the 80 children who had malaria only were subjected to cytokines concentration determination using commercial standard enzyme linked immunosorbent assay kits (Abcam, UK). **Results**: Eighty participants with co – infection had a mean malarial parasitaemia of 662 ± 201.1 μL while the 80 participants with only P. falciparum malaria had a mean malarial parasiteamia of 5943 ± 3270.7μL. Also the volunteers had mean haemoglobin of 11.2 g/dL for co – infected individuals and 5.7 g/dL for participants with single infection of malaria. The serum cytokine levels of the children with S. haematobium and P. falciparum and only P. falciparum infection are as follows; interleukin – 4 (16.6 pg/mL versus 5.2 pg/mL), IL – 5 (501.3 pg/mL versus 357.5 pg/mL); IL – 8 (2 550 pg/mL versus 309 pg/mL), IL – 10 (273 pg/mL versus 290 pg/mL), TNF – α (25 pg/mL versus 290 pg/mL) and IFN – γ (21.9 pg/mL versus 2.5 pg/mL). The TNF – α /IL – 10 ratio is 7 for the children with co – infection while those with only P. falciparum malaria infection had a TNF – α/IL – 10 ratio of 0.9. **Conclusion**: We conclude that the elevated IL – 4, IL – 5, IL – 8 and IFN – γ concentration induced by schistosomiasis altered the Th1/Th 2 profile and protected the children against the morbidity and severity of malaria attack among the children with co – infection.

Keywords: Schistosoma haematobium; Plasmodium falciparum; Malaria

INTRODUCTION

With more than 1 million child deaths annually, malaria remains the single leading killer of children in sub-Saharan Africa[1]. Among parasitic diseases, schistosomiasis is considered to be second to malaria in global importance[2]; and studies among affected populations have shown highest prevalence and intensity of *S. haemaiobium* infection in school-age children[3,4]. Polyparasitism is common in tropical areas of which interactions between parasites have been suggested[5-8]. Co-infection of malaria and

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schistosomiasis has been reported to exacerbate disease morbidity[9].

The implication of concomitant malaria and helminths interactions have been mainly explored in animals under laboratory conditions[10-12]. In human populations, only few studies have been conducted with contradictory results of protective role of helminths[13-15] and deleterious effect of co- infection[14, 16].

Anaemia is one of the widespread and common health conditions afflicting individuals living in the tropics especially in Africa[17, 18]. Impairment in physical growth, cognition and school performance are consequences of severe anaemia in children[19, 20]. Although the etiology of anaemia is complex and multifactorial in origin, parasitic diseases, including *P. falciparum* and helminth infections have long been identified as major contributors to anaemia in endemic countries[17, 21]. In fact, the occurrence of anaemia and other complications in *P. falciparum* malaria as well as the protective effects arising from co-infection of these parasitic infections have been elucidated immunologically using cytokines profile from different parts of the globe[22-25].

In spite of the risk associated with concomitant infection of Plasmodium and helminth parasites[26] and the contradictory *Plasmodium*-helminths interactions reported in different geographical areas[13, 14, 16]; information on the impact and relationship that exists between *P. falciparum* and *S. haematobium* co-infection is lacking in our locality. This study therefore reports co-infection of malaria and schistosomiasis in some Nigerian children. Also the haemoglobin profile of the participants and the protective effects of the association of these parasitic infections using cytokines profile were investigated.

MATERIALS AND METHODS

This study was carried out in Ihieve-Ogben in Edo State. Ihieve - Ogben is a schistosomiasis endemic rural community. The studied area is located at latitude 6°N and longitude 6°E. Malaria transmission is perennial but highest during the rainy season. The rainy season period is between April and October while the dry season period is between November and March.

This investigation commenced during a community mobilization campaign at Ihieve-Ogben. This involved educating them on the significance of the study, all procedures as well as seeking their consent. They were recruited after informed consent was obtained. Ethical permission was obtained from the State Ministry of Health, Benin City, Nigeria. Mid stream urine samples were collected from 80 children who had history of haematuria and occasional low grade febrile condition between 11:00 and 13:00 GMT after slight physical exercise. The specimen was kept in a wide-mouthed screw capped 50(mL) size container. These bottles containing the urine samples were immediately transported to our parasitological laboratory for examination for the ova of *S. haematobium*. The ova were quantified and classified as light infection $\leqslant$50 ova/10 mL and heavy infection $>$ 50 ova/10 mL according to WHO standards[27,28].

Blood samples were collected from the 160 comprising the 80 children with schistosomiasis and *P. falciparum* co-infection and the 80 children who had malaria. The positivity for malarial attack was based on *P. falciparum* parasitaemia in their thick blood smears stained by Geimsa stain. The malaria parasitaemia was categorized as uncomplicated ($<$ 10 000 parasite/μL) and severe ($>$10 000 parasite/μL). They also had fever (axillary temperature of $>$37.5℃). Clinical symptoms such as headache, vomiting, diarrhea, prostration, respiratory distress and other symptoms and signs of severe malaria were found among these volunteers as documented by[29]. The volunteers with other overt diseases such as measles, respiratory tract infections, HIV using standard kits were excluded from the study.

Whole blood samples were obtained from the volunteers and heamoglobin profile of the participants was ascertained using standard laboratory procedure. The blood was processed and the serum was subjected to cytokines concentration determination using commercial standard enzyme linked immunosorbent assay kits (Abcam, UK) according to the manufacturer's instruction.

The data obtained in this study were subjected to statistical analysis, namely, t-test and chi-square test using Microsoft Excel statistical package.

RESULTS

Table 1 showed the prevalence of co-infection of *S. haematobium* and *P. falciparum* malaria matched with those with only *P. falciparum* infection, their malarial parasitaemia and their heamoglobin status according their age groups. The children with the co-infection had a mean malarial parasiteamia of 662 ±

201.1 μL while those with only malaria had a higher parasitaemia of 5 943.1 ± 3 270.1 μL. This difference was statistically significant ($\chi 2 = 4\,230$, $P < 0.05$). The mean haemoglobin level of 11.2 g/dL was recorded for the children with co - infection while those with only *P. falciparum* infection had mean haemoglobin of 5.7 g/dL. This difference was not statistically significant at ($\chi 2 = 0.85$, $P < 0.05$).

Children with light *S. haematobium* infection (≤50 ova/10mL urine) had malaria parasitaemia of 480 ± 256.2 μL while the malarial parasitaemia for heavy *S. haematobium* infection (>50 ova/10 mL urine) was 843.3 ± 311.2 μL. This difference was statistically significant at ($\chi 2 = 99.8$, $P < 0.05$) (Table 2).

The mean serum cytokines concentration for the two categories of children with *S. haematobium* and *P. falciparum* co-infection and only *P. falciparum* malaria is presented in table 3. The mean cytokines concentration of these groups of children are IL- 4 (13.5 pg/mL versus 3.5 pg/mL), IL-5 (501.3 pg/mL versus 357.5 pg/mL), IL-8 (1 971.3 pg/mL versus 309 pg/mL) and IFN-γ (8.5 pg/mL versus 2.5 pg/mL). The differences between these cytokines were statistically significant at ($\chi^2 = 5.8$, $P < 0.05$; $\chi^2 = 24.1$, $P < 0.05$; $\chi^2 = 1211$, $P < 0.05$; $\chi^2 = 3.2$, $P < 0.05$), respectively. The malarous children had a statistically significant higher mean TNF-α (290 pg/mL) than those with co-infection (25pg/mL) and this difference was statistically significant ($\chi^2 = 222.9$, $P < 0.05$). The TNF-α / IL-10 ratio for the children with co-infection was 7 while those with *P. falciparum* only had a TNF-α / IL-10 ratio of 0.9.

Table 1 The intensity and prevalence of *S. haematobium* and *P. falciparum* co-infection according to the different age group of the children.

Age group in years	Co-infection with *P. falciparum and S. haematobium*			*P. falciparum infection*		
	Number (%)	Malaria parasitaemia /μL	Heamoglobin level (g/dL)	Number (%)	Malaria parasitaemia/ μL	Haemoglobin Level (g/dL)
1-5	20(20.5)	450 ±312.2	11.2 ±2.1	20	8900 ±988.5	4.0 ±2.2
6-10	34(42.5)	850 ±259.8	11.9 ±1.9	34	6499.3 ±1417.7	4.5 ±1.8
11-15	26(32.5)	686 ±209.6	10.6 ±1.5	26	2430 ±2009.2	8.2 ±2.9
	80	662 ±201.1	11.2 ±0.9	80	5943.1 ±3270.7	5.7 ±2.3

Table 2 Intensity of Schistosoma haematobium infection and malaria parasitaemia.

Intensity of *S. haematobium* infection	Number infected	Malaria parasitaemia
Light infection(≤ 50 ova/mL)	58	480.0 ±256.2
Heavy infection(>50 ova/10mL)	22	843.4 ±311.2
	80	661.0 ±257.0

Table 3 The mean serum cytokines profile of the participants with *S. haematobium* and *P. falciparum* infections.

Cytokines	Mean Cytokines levels of participants with *S. haematobium* and *P. falciparum* infections (pg/mL)	Mean cytokines levels of malarous participants (pg/mL)
IL-4	13.5 ±5.2	3.5 ±2.6
IL-5	501.3 ±156.0	357.5 ±113.8
IL-8	1971.3 ±418.6	309.0 ±245.3
IL-10	175.5 ±142.9	272.8 ±133.1
TNFα	25.0 ±10.5	290.0 ±105.4
IFNγ	8.5 ±1.9	2.5 ±0.9

DISCUSSION

We reported lower parasitaemia for co-infected children over children infected with *P. falciparum* only. This result corroborates the finding of[24]. This demonstrates helminth-associated protection against malaria. We hypothesize that this protective role of helminth against malaria is immunologically mediated. Schistosomes have a dorminant Th2 biased cytokine responses related to egg production[30]. Chronic immune activation due to helminth infection may cause altered responses to secondary stimulus such as ma-

laria parasite that depends upon Th1 cytokine production[31, 32] and may also alter T-cell memory responses[33]. Thus, the Th2 cytokines (IL-4 and IL-8, IL-5 and IFN-γ) elevated in schistosomiasis and malarous co-infected children compared to uninfected children play significant role in modulating the host responses to malaria infection[24]. This indicates an imbalance in the Th1/Th2 responses with the down regulation of the Th2 responses critical in the *P. falciparum* malaria.

We observed that mean haemoglobin level for children with malaria infection only was lower than children with malaria and schistosome co-infection. This supports the findings of Brooker et al[34] and Stephenson et al[35] who reported lower haemoglobin concentration for both preschool and school-age Kenyan children for co-infection of *Plasmodium* and helminth parasites than single infection of either *Plasmodium* or helminth parasite. These results show an additive impact of co-infection on anaemia and implicate the immunologically mediated protective role of schistosome in reducing malaria pathology[36]. This no doubt reflects the impact of the pathogenesis of these aetiological agents on the participants. For instance malaria contributes to the reduction in haemoglobin concentrations by destruction and removal of parasitized red cells and the shortening of life span of non parasitized red cells and decreasing the rate of erythrocyte production in the bone marrow[37]. Furthermore, some anaemia caused by malaria are associated more with acute clinical states (e.g., hemolysis or cytokine disturbances), whereas chronic or repeated infections are more likely to involve dyserythropoiesis[38]. Schistosomes also cause anaemia by chronic blood loss, as eggs penetrate the urinary tract[21].

Also of immunopathological interest is the pattern of the IL-10/TNF-α ratios which can in part be responsible for the anaemia profile of these two major groups of participants where those malarous patients with more severe anaemia had a lower IL- 10/TNF-α ratio than their counterparts with moderate anaemia and high IL-10/TNF-α ratio. This observation accords the reports of[23, 29, 39] where they attributed the associated anaemia to low IL-10/ TNF-α. Also the net effect is a loss of the counter regulatory antinflammatory function of the IL-10. This anaemia profile can be attributed to the high TNF-α level recorded among the malarous patient. This observation had been documented earlier[22] where they reported that TNF-α contributes to anaemia by erthrophagocytosis and dyserthrpoiesis.

The elevated IFN-γ level reported among those with co-infection corroborates the observation made by[24]. As advanced earlier, the CD8 + demonstrated to be critical in the granulomatous response to egg deposition[25] and protective effects on *S. haematobium* infection[40] may secrete IFN-γ in response to ova induced IL-4 production which is elevated in our studied participants with co-infection. This contributes to the down regulation of Th2 production which is critical for immunopathology of *P. falciparum* malaria.

In conclusion, it was observed that malaria parasite density in children decreased with age. This result is expected and has been attributed to be due to specific anti-malarial immunity that develops in disease endemic areas with repeated infections[24]. Our results also showed that haemoglobin levels were higher in children with co-infection than children with only malaria infection. Also we conclude that the elevated IL-4, IL-5, IL-8 and IFN-γ concentration induced by schistosomiasis altered the Th1/Th 2 profile and protected against the morbidity and severity of malaria attack among the children with co-infection. Therefore, the *S. haematobium* infection confers protection for participants who were co-infected with *P. falciparum* malaria.

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