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Subtle morbidities associated with malaria co-infection with schistosomiasis among children in South-West Nigeria.

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Abstract

Background: Malaria co-infection with schistosomiasis is known to modulate the immune response and thereby to potentially alter the pathophysiological and immunological profile of the diseases. The aim of the study was to determine the relationship between subtle morbidities and co-infection with malaria and schistosomiasis, and the immunological responses to the two diseases, among children in rural southwest Nigeria.
Methods: A cross-sectional survey was conducted between April and July 2012 among primary and secondary school children in Eggua, Yewa North LGA, Ogun State and Omi-Adio, Iddo LGA, Oyo State. A total of 240 children (Yewa 91, Iddo 149) participated in the study. Blood and urine samples were collected from the children and analysed by microscopy for *Plasmodium falciparum* and *Schistosoma haematobium* respectively. All the samples were analysed for IL-10, IFN-γ, and some for antibodies to *Plasmodium falciparum* $MSP1_{19}$. Packed cell volume (PCV) and some anthropometric indices (height, weight) were measured as indicator of subtle morbidities of infection with the two parasites.
Results: The prevalence of co-infection with the two parasites in the study was 16%. Malaria prevalence was 35.6% in Eggua, 20.13% in Iddo, and highest in the 11-15yr age group. Average malaria parasite density was 195.67 parasites/µl blood. Schistosomiasis prevalence was 20.8% in Iddo, 30.8% in Eggua, with highest intensity of infection in age group 11-15 years in both areas. Anaemia was not prevalent among co-infected people (16%). Antibodies to $MSP1_{19}$ were found in 36.7%. Peripheral IL-10 levels did not differ significantly among malaria, schistosomiasis, or co-infected individuals, but IFN-γ was higher among older children with schistosomiasis.
Conclusion: Anaemia was not a very discriminating index to indicate morbidity from the diseases in this study area.

Keywords: *Mobidities, co-infection, Schistosoma, Plasmodium falciparum, cytokines*

Résumé

Contexte: La co-infection avec le paludisme schistosomiase est connu pour moduler la réponse immunitaire et ainsi potentiellement à modifier le profil physiopathologique et des maladies immunologiques. Le but de l'étude était de déterminer la relation entre morbidités subtiles et co-infection par le paludisme et la schistosomiase, et les réponses immunologiques aux deux maladies, parmi les enfants du sud-ouest Nigeria rural.
Méthodes: Une enquête transversale a été menée entre Avril et Juillet 2012 Parmi primaires et secondaires écoliers de Eggua, Yewa Nord LGA, Etat d'Ogun et Omi-Adio, Iddo LGA, l'Etat d'Oyo. Un total de 240 enfants (Yewa 91, Iddo 149) ont participé à l'étude. Des échantillons de sang et d'urine ont été prélevés sur les enfants et analysés par microscopie pour Plasmodium falciparum et Schistosomahaematobium respectivement. UNE
ll les échantillons ont été analysés pour l'IL-10, IFN-γ, et d'autres pour les anticorps dirigés contre Plasmodium falciparum $MSP1_{19}$. Le volume de l'hématocrite (PCV) et certains indices anthropométriques (taille, poids) ont été mesurées comme indicateur de morbidités subtiles d'infection avec les deux parasites.
Résultats: La prévalence de la co-infection avec les deux parasites dans l'étude était de 16%. La prévalence du paludisme était de 35,6% en Eggua, 20,13% dans Iddo, et le plus élevé dans le groupe d'âge 11-15yr. La densité moyenne du parasite du paludisme était 195,67 parasites / µl de sang. la prévalence de la schistosomiase a été de 20,8% en Iddo, 30,8% en Eggua, avec la plus grande intensité de l'infection dans le groupe d'âge 11-15 ans dans les deux zones. L'anémie était pas répandue chez les personnes co-infectées (16%). Les anticorps anti- $MSP1_{19}$ ont été trouvées dans 36,7%. Périphériques IL-10 niveaux ne diffèrent pas significativement entre le paludisme, la schistosomiase, ou des personnes co-infectées, mais IFN-γ était plus

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élevé chez les enfants plus âgés atteints de schistosomiase.
Conclusion: L'anémie était pas un indice très discriminant pour indiquer la morbidité des maladies dans cette zone d'étude.

Mots-clés: *Mobidities, la co-infection, Schistosoma, Plasmodium falciparum*

Introduction

Malaria and schistosomiasis are the most prevalent tropical diseases in sub-Saharan Africa and together exert a huge burden of mortality and morbidity. Under-recognized, 'subtle' morbidities such as caloric malnutrition, growth stunting, anaemia, and poor school performance are significant correlates of both helminthic and protozoan parasitic infections. The geographical overlap of these two human parasites *Schistosoma haematobium* and *Plasmodium falciparum*, and the prevalences of both parasites in exposed individuals rise with age, peaking in childhood and this overlap results inevitably in frequent co-infections [1,2]. Long standing infection from schistosomiasis leads to chronic inflammation which results in subtle morbidity. The most important are anaemia, iron deficiency anaemia, growth stunting, malnutrition, fatigue and diminished physical fitness. Impaired cognitive development and hepatosplenomegaly can also result from chronic infection with schistosomiasis [3]. These under-recognized effects of schistosomiasis usually have the most telling effect on the human capital of endemic communities. The indirect effects of infection may result in poor school performance and limited job performance, and certainly affect the overall development of local communities hence leading to poverty [4].

During acute malaria, hepatosplenomegaly also occurs due to reticuloendothelial and lymphoid hyperplasia. As the parasitaemia increases or fails to clear, hepatosplenomegaly becomes persistent; but as immunity to malaria develops, the time between malaria attacks increases and a corresponding drop in the prevalence of hepatosplenomegaly occurs [5]. Malaria causes anaemia by the destruction and removal of parasitized red blood cells and shortening of the life span of non-parasitized red cells, and decreasing the rate of erythrocyte production in bone marrow [6].

Malaria due to *Plasmodium falciparum* (*Pf*) remains one of the major public health issues in tropical countries and the vast majority of childhood deaths due to malaria occur in sub-Saharan Africa [7]. Depending on the intensity and duration of the individuals' exposure to the parasite, protective immunity to *Pf* malaria is slowly acquired after several infections [8]. Immunity to malaria and schistosomiasis is known to develop gradually with age, with younger individuals of endemic populations being at a higher risk of clinical malaria than older ones [9, 10]. In infected humans, humoral immune responses to blood stage parasites play a primary role in providing protection against malaria [11] and they are largely dependent on cytophilic type immunoglobulin (Ig) antibodies (Abs) such as IgG1 and IgG3 isotypes. In *Plasmodium* infection IgG1 and IgG3 antibody subclasses are associated with protection against merozoite surface protein (MSP) antigen [12-14]. In addition, a specific cellular immune response and its associated cytokines, play a key protective or pathological role during malaria. Some cytokines, such as interferon-γ (IFN-γ and Interleukin-10 (IL-10) are known to be directly involved in the production of specific isotypes of anti-*P. falciparum* antibody responses. It was recently demonstrated that in Ghanaian school children, there was an increase in specific IL-10 production in helminth-infected individuals, compared to non-helminth infected [15].

Schistosomiasis is second only to malaria as a parasitic disease of serious public health importance in subtropical and tropical Africa. It is typically a disease affecting agricultural communities, particularly those dependent upon irrigation to support their agriculture; and also in communities facing the challenge of potable water supply. Children of school age are especially at risk because of their daily contact with infected water in rural areas. Current evidence suggests that schistosomiasis, although rarely lethal, has significant impact on multiple dimensions of human performance both during childhood and later adult life including anaemia, impaired cognitive development, intellectual and physical development [16]. Schistosomes cause anaemia by chronic blood loss, as eggs penetrate the wall of the bowl (in intestinal schistosomiasis) and the urinary tract (in urinary schistosomiasis) [17, 18]. Studies on *Schistosoma haematobium* indicate that the balance between adult schistosome-specific IgE and IgG4 is one of the key indicators of the development of protective immunity to the infection while anti-cercariae IgE and IgG4 responses are associated with hypersensitivity which in turn causes cercarial dermatitis [19, 20].

Malaria co-infection with schistosomiasis is known to modulate the immune response thus potentially alter the pathophysiological and immunological profile

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of the disease [21]. The pathophysiology of both parasitic infections is immune-mediated, such as cerebral malaria or schistosome granuloma and fibrosis [22].

The present study was conducted to determine the impact of malaria and schistosomiasis on children, to investigate the immunological involvement in the subtle morbidities and measure the antibody and cytokine responses of children co-infected with malaria and schistosomiasis.

Materials and methods

Study area

The study areas were Ologuneru and Omi-Adio both in Iddo LGA Oyo State, and Eggua village in Yewa North LGA of Ogun State. As estimated by the 2006 population census, Iddo Local Government Area had a total population of 53,582. The people of Iddo are mainly small scale farmers with a significant proportion of the farmers engaging in a secondary occupation such as hunting, trading, artisanry, civil service jobs. Farmers in the area grow mainly food crops such as maize, cassava, yam and vegetables. They also engage in the cultivation of some cash crops like cocoa, kola and oil palm.

Eggua is situated in Yewa North Local Government Area (LGA), Ogun State and lies between latitude 7° 15 N and longitude 3° 3 E in a deciduous or derived savannah zone with a land size of about 215 Km^2. The area is dominated largely by Yoruba speaking people [23]. Trading, timber logging and farming are the main occupations of the inhabitants. Both local government areas lie within the rainforest of sub-Saharan Africa and two notable seasons occur; rainy and dry seasons that varies from April to September and October to March, respectively. Malaria and Schistosomiasis are endemic in both areas; transmission occurs year round and it peaks during the rainy season when there is much exposure to mosquitoes and frequent contact with water bodies containing the snail hosts.

Study design

The aim of the study and the protocols involved were explained in English and Yoruba to the children in various schools of the communities. General information regarding the nature of the study and its objectives was also explained to teachers, parents or guardians. Simple random sampling was used to select children to participate in the study. Using the attendance registers of the participating schools in Iddo, and tables of random numbers, the selection of the children to participate in the study was achieved. Confidentiality and anonymity were maintained throughout the study period. Inclusion of children into study took place after written and verbal informed consent had been received from school authorities and parents. Children joined the study voluntarily and were free to drop out of the study at any time.

The sample size was 246, estimated according to Naing *et al.*, [24]. For this study we assumed expected prevalence to be 80% (0.8) and precision at 0.5, which is an assumption of a normal approximation. Each child provided information on their place of residence, water usage and water contact activity; the frequency of visible haematuria was also noted and recorded. Blood samples were collected intravenously from each child by trained personal for further assay in the laboratory while schistosome- infected participants were treated with a single dose of Praziquantel at 40mg/kg body weight.

Ethical consideration

Ethical clearance for the study was given in compliance with the approved ethical guidelines laid down by the UI/UCH ethical committee. A pre-survey visit was made to the study area during which time, discussions were held with the Community Heads and school teachers who assisted in mobilizing the pupils for the study. Written informed consents were obtained from those willing to participate in the study. Results were treated with confidentiality.

Sample collection and storage

Blood

Blood (2ml) was collected into sterile ethylene-diamine-tetraacetic acid (EDTA) sample bottles and kept on ice. Blood samples were centrifuged in the laboratory at 3000 revolution per minutes (rpm) for 5 minutes; plasma samples recovered were stored at -80°C. Blood samples were also collected into a Sodium-heparinized micro-haematocrit capillary tube and sealed at one end and then centrifuged at 1500rpm for 5 minutes. A Hawksley micro-haematocrit reader was used to measure the packed cell volume (PCV).

Parasitemia

Thick films were made and stained with 1% Giemsa stain for thirty minutes, washed off and allowed to air dry. The slides were examined under the x100 objective lens. Parasitaemia was determined by three-time count

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of 200 white blood cells (WBC) and malaria parasites on every field.

Urine

Urine samples were collected between 10:00 and 14:00 hours in transparent 20ml universal bottles. 10 ml of urine was transferred into a centrifuge tube and spun for 5 min at 1500 rpm. The supernatant was discarded and a drop of the sediment was placed on a clean grease-free slide and covered with a cover slip. It was then examined microscopically using the 10x objective for the presence of terminal-spined ova of *Schistosoma haematobium*. A positive sample was indicated by the presence of ova of *S. haematobium* and expressed as number of eggs/10ml of urine. A negative sample was indicated by the absence of parasite eggs.

Determination of anti-meroziote surface protein-1_{19} total IgG by Elisa

The serum samples were analyzed by ELISA for P*lasmodium*-specific total IgG to demonstrate the responses of the children to MSP-1-19 recombinant antigen. Flat bottom 96-well polystyrene plates (Corning Incorporated- Life Sciences, MA, USA) were coated with 100ìl of coating buffer (using a concentration of 197ìg/ml in carbonate-bicarbonate buffer) and then incubated overnight at 4 °C. The antigen was then poured off and blocked with 200ul of 10% Skimmed milk/ PBS-Tween-20 for an hour at 37°C in an incubator. The plates were then washed three times with PBS/Tween- 20. Sera were then diluted 1:100 in 5% Skimmed milk and 100ul of the dilution was added to each well in duplicate and incubated for another hour at 37°C. The plates were washed three times with PBS/Tween- 20 and 100ìl of Horseradish Peroxidase anti-human immunoglobulin G conjugate was added per well at a dilution of 1:2000 and then incubated at 37 °C. Finally the plates was washed three times with PBS-Tween- 20, colorimetric development was allowed by the addition of 100µl per well of substrate solution; ABTS (2,22 -azino-bis (3-ethylbenzothiazoline 6-sulphonic acid) diammonium; Sigma) in 50 mM citrate buffer (pH =4 containing freshly prepared 0.003% H_2O_2). The reaction was allowed to proceed in the dark for about 30 minutes and the absorbance was then measured at 405 nm with a microplate reader without stopping the reaction. The assay included a reference negative control.

Measurement of cytokines in malaria and schistosomiasis patients by elisa

The serum levels of cytokines (IL-10 and IFN-γ) in children with malaria and schistosomiasis infections were determined using ELISA kits (Mabtech, Stockholm. Sweden). The assay was performed in 96-well microtitre plates (NUNC-Immuno Plates, Denmark) using the protocol developed by MABTECH with slight modification. First, the wells were coated 100µl/well with high affinity monoclonal antibodies (mAb 1-D1K) diluted to 5µg/ml in Phosphate Buffered Saline (PBS) (pH 7.4) and incubated overnight at 4°C. Unbounded monoclonal antibodies were poured off and plates were blocked 200µl/well with 0.05% Tween-20 diluted in PBS (PBS-Tween20) supplemented with 3% Skimmed milk and incubated for one hour at room temperature. Plates were then washed five times with PBS-Tween20. Standards (hIL-10 and hIFN-ã) were serially diluted in incubation buffer (1% Skimmed milk/ PBS-Tween20) from standard hIL-10 stock solution of 1µg/ml and standard hIFN-ã stock solution of 10µg/ml. Samples were also diluted at 1:200 in incubation buffer. 100µl/well of samples and standard were added to the different plates (for standard curve and test plates) and incubated overnight at 4°C. Washing was repeated five times, then 100µl/well of 1:200 dilution of 2.5µg/ml IL-10 12G8-biotin and IFN-γ 7-B6-1-biotin detection monoclonal antibody were added to plates and incubated for one hour at room temperature. After washing five times, 100µl/well of Streptavidin-HRP (Horse-radish Peroxidase) conjugate diluted 1:1000 in incubation buffer was added to plates and incubated for one hour at room temperature. Washing was repeated and 100µl/well of ABTS (2,22 -azino-bis (3-ethylbenzothiazoline 6-sulphonic acid) Sigma) in 50 mM citrate buffer (pH=4 containing freshly prepared 0.003% H_2O_2) substrate solution was added. Avidin-ALP (Alkaline Phosphatase) conjugate diluted 1:1000 in incubation buffer was also used with BCIP used as the substrate solution. The reaction was allowed to proceed in the dark for about 30minutes and the absorbance was then measured at 405nm with a microplate reader without stopping the reaction. The assay included a reference negative control.

Data analysis

Data entry was done using Microsoft 2007 excel and SPSS version 16.0 for Window Prevalence of infections and 95% confidence interval (CI) were calculated. Descriptive statistics including percentages and mean values were used to analyze data obtained through questionnaire. Correlations were analysed with

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Spearman's correlation. Frequencies and percentages were used to present categorical variables. Cross tabulations and Chi-square tests were used to determine dependence between categorical characteristics. P-values less than 0.05 were considered statistically significant.

Results

An overall prevalence of 24.6% for schistosomiasis was found among the study subjects:- 20.8% (31) of the cases in Iddo LGA and 30.8% (21) in Eggua in Yewa North. About 31 (29.8%) males and 28 (20.6%) females in the study were infected p>0.05. Most of the positive schistosomiasis cases were distributed into the 11-15 age-group. The intensity of *Schistosoma* infection by egg counts was low among the females but there was heavy infection among the male (>50 eggs/10ml urine). The highest intensity was among children in the age group of 11-15, and there was no significant association between the intensity, age and sex.

Table 1: Intensity of Schistosomiasis in the sample population

No infection	181(75.41%)
Light	
1-9 eggs/10ml	23(8.33%)
Moderate	
10-49 eggs/10ml	20(8.33%)
Heavy	
>50 eggs/10ml	16(6.67%)

PCV: Anaemia determination

Values for packed cell volume (PCV) were normal in a majority of the study subjects with an overall frequency rate of 88.3% while 11.7% were anaemic (i.e PCV = 33% - 38%) . According to the WHO standard, mild anaemia is when haematocrit is <33%, severe anaemia when haematocrit is <15%. A total of 11.5% anaemia occurred among males and 11.8% among females, while 9 children (20.5%) in the age range of 5-10 had anaemia, 16 children (12.5%) in the age range of 11- 15, and 3 (4.4%) among those that were 16 and above had anaemia. Among individuals infected with malaria, 16.4% were anaemic, while 3.4% of those infected with schistosomiasis and 5% of those co-infected were anaemic (Figures 1 & 2).

Anti-MSP-1_{19} antibody levels

Anti-MSP-1_{19} antibody titre was high among children between the ages of 16 and above followed by children in the age range of 11-15. The antibody titre value was low among children between ages 5 to 10. The antibody titre showed a strong significant correlation with increasing age, (P-value = 0.013).

Prevalence of Malaria Co-infection with Schistosomiasis

A positive diagnosis for malaria was made for 67 (27.9%) cases of the study population. 12.5% of them were male (30) and 15.4% were female (37). 8.8%

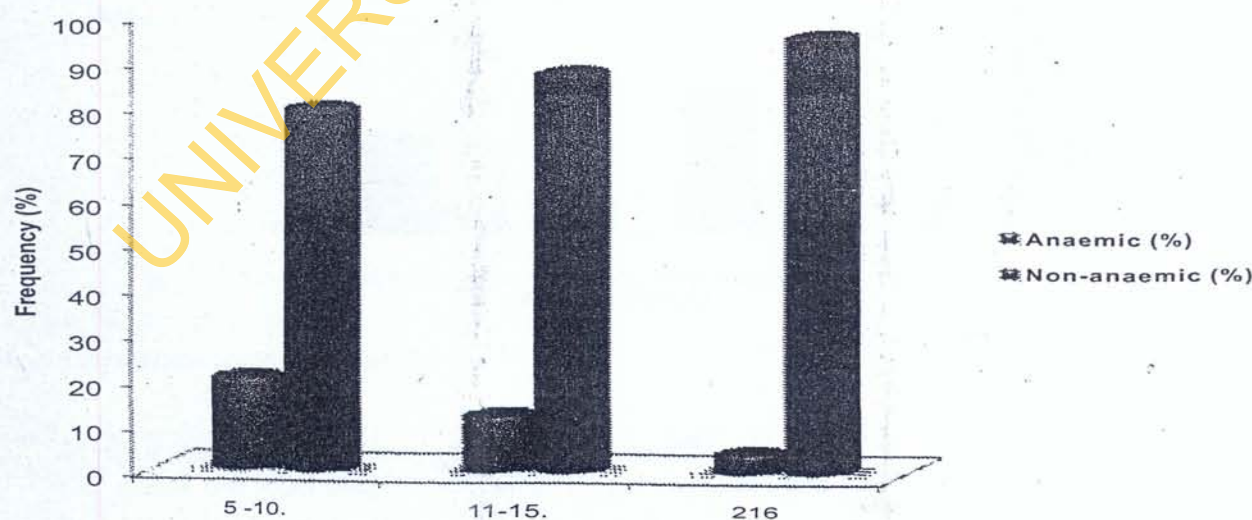

Fig.1: Frequency Ratio of Anaemia in the study population with respect to age.

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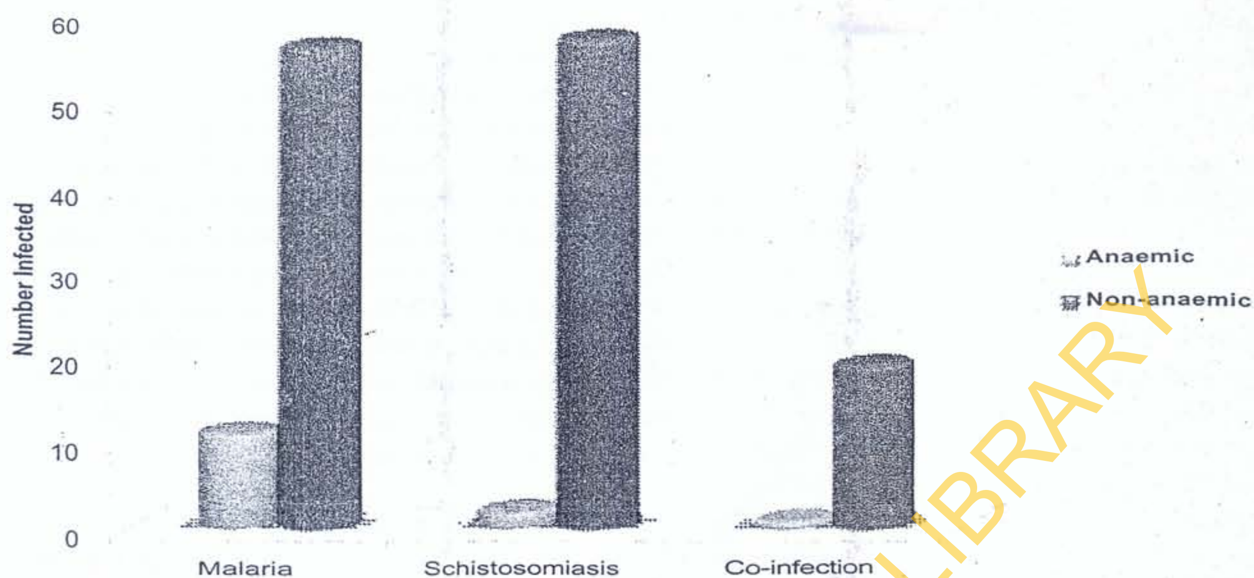

Fig. 2: Frequency ratio of anaemia among infected individuals.

Fig. 3: Prevalence of infections in relation to age.

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(21) of the population was between the age group of 5-10 years, 14.2% (34) are between age 11-15 and 5% (12) was between age 16 and above. Most of the positive malaria cases were seen among children between ages 11-15. An overall prevalence of 24.6% for schistosomiasis was found among the study subjects-20.8% (31) of the cases in Iddo LGA and 30.8% (21) in Eggua in Yewa North. Most of the positive schistosomiasis cases were in the 11-15 age-group. Above all, twenty children (8.3%) among the study

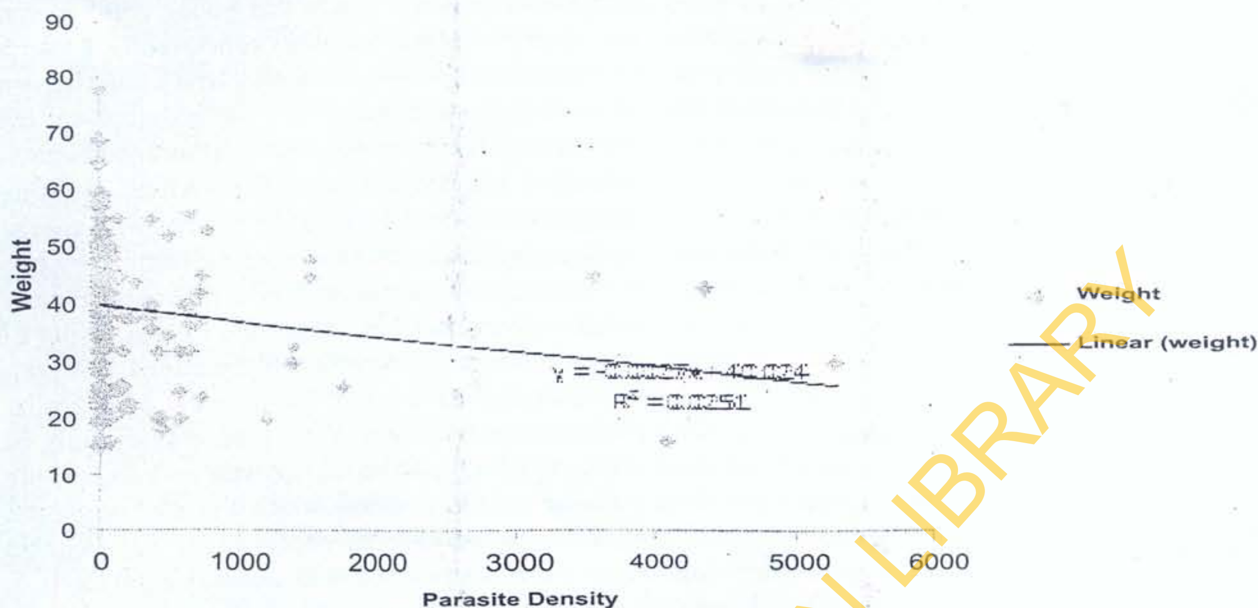

Fig. 4: Body weight correlated to parasite density.

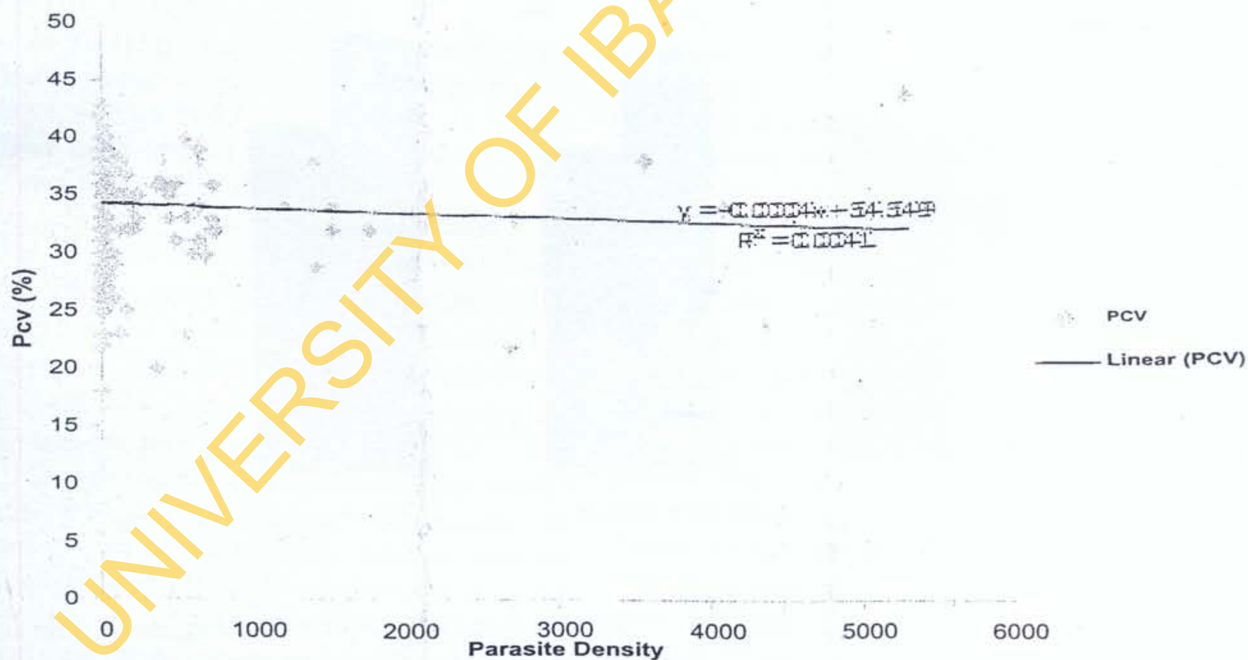

Fig. 5: Parasite density correlated with PCV.

population were co-infected: 11 (10.6%) of them were males and 9 (6.6%) were females. Among the age groups, the highest prevalence (9%) was found in those between the ages of 5-10 (Figure 3). Co-infection risk for schistosomiasis in the sample population was 0.755 (95% CI= 0.477- 1.196).

Correlation between malaria parasite density, weight and PCV

There was a positive correlation between malaria parasite density, body weight and packed cell volume (PCV). These showed that for every individual with parasitaemia of 51-500 parasites/µl blood the PCV was

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between 30% and 40%. These implies that there was a significant association between PCV and parasite density, where P<0.05 (P-value= 0.054). There was also a significant association between the parasite density and the body weight where P = 0.013 at P <0.05 level (Figures 4 & 5).

Cytokine levels in study population

The standard curve for both IL-10 and IFN-ã showed a simple linear relationship between the optical density and (OD) and the concentration of recombinant human IL-10 and IFN-ã standards for the avidin-HRP conjugate used. The cytokine levels for both IL-10 and IFN-ã in the samples tested ranged from 0.054 to 0.199 (OD) for samples avidin-HRP conjugate was used while for those samples avidin-ALP conjugates was used the optical density ranges between 0.100 and 0.900. Level of IL-10 in the study population was higher than that of IFN-ã with respect to age, sex, and infections (Figure 6).

Schistosoma haematobium and *Plasmodium falciparum* in Africa means that young children can be infected with both parasites concurrently. Malaria co-infection with schistosomiasis exerts a huge burden of morbidity and mortality and the geographical the overlapping distribution of *P. falciparum* and helminth infections is common in sub-Saharan Africa, resulting in high rates of co-infections [2]. Studies with animal models have shown that concurrent infections by two or more parasite species could affect the pathogenesis of each other. They have suggested the possibility of antagonistic or synergistic interactions between parasites, and have raised the question of a similar phenomenon in humans. In the present study, only 20 children (8.3%) in the population were co-infected with malaria and schistosomiasis, which is in close agreement with the prevalence as observed by [2, 30]. No significant association was observed between *S. haematobium* and asymptomatic *P. falciparum*

Fig. 6: Cytokine levels of infected individuals in Eggua

infections, this is in conflict with the results of a previous study [31] but in agreement with another study from Uganda that reported no association [32].

Discussion

This study provided preliminary data on prevalence of malaria and schistosomiasis co-infection and also the cytokine levels in Iddo LGA and Eggua, Yewa North LGA. It also provided a basis for further immunological studies to determine the relationship between subtle morbidity, antibody and cytokine response to co-infection in areas known to be endemic. The geographical and demographical co-distribution of

In this study, the overall schistosome infection prevalence was 24.6%, with no significant difference between the two study areas. Prevalence was higher in males (29/8%,) than females (p = 0.100, X^2= 2.702). This could be as a result of their frequent contact with the water-bodies for recreation (to swim) as well as work (farming and fishing) both in the rainy season and

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dry season (irrigation). There was no significant association between gender and prevalence of infection $p > 0.05$. Intensity of infection increased with age as also found in other studies [23]. Children between the age group of 11 and 15years had parasite intensity reflecting light, moderate or heavy infection (Table 1). This is in close agreement with [25] where intensity both in adult and children ranged from 11-33%. The distribution of schistosomiasis among the children based on age and sex was similar to the report of [26].

The overall prevalence of malaria in the study was low 67(27.9%) and not significantly associated with gender. There was an association between malaria prevalence and age, ($p = 0.001$). Children between the ages of 11 and 15 years had the highest parasite density values of 500 parasites/µl of blood. In many malaria sero-epidemiological studies, high titres of antibodies to antigens are often associated with protection [13], though others have argued that antibody quantity does not necessarily mean antibody quality with respect to protection. Similar to malaria, immunity to schistosomes takes several years to develop to levels sufficient to have observable effects on either infection or re-infection [10].

In the study population, IL-10 was the more prevalent cytokine, except among the children aged 16 and above, where IFN-ã levels were high. The higher prevalence in the level of IL-10 is consistent with the study reported by Milner *et al.*, [27] indicating that levels of both schistosome-specific and systemic IL-10 were low. The high prevalence of IL-10 may be related to its role in isotype switching to IgG subclasses and its regulatory function of modulating both Th1 and Th2 responses [28, 29]. The least frequently detected cytokine was IFN-γ although it was high among the children between ages 16 and above (Fig. 6).

Chronic schistosomiasis causes subtle morbidity, such as under-nutrition and anaemia, the importance of the subtle morbidity has only recently been acknowledged [4]. Under-nutrition and anaemia have detrimental effects on morbidity, mortality, cognitive development, reproduction and physical work capacity; subsequently, they affect not only children, but continue to have an impact on them far into adulthood [33].

The causes of both under-nutrition and anaemia among school-age children and adolescents in the developing world are multi-factorial and are largely driven by poverty. Major determinants of under-nutrition include a history of under-nutrition (that is, poor prenatal nutritional status and under-nutrition during infancy and early childhood), deficient dietary intake due to inadequate food resources and recurrent or chronic infections leading to anorexia, mal-absorption, gastrointestinal loss of nutrients, and the release of pro-inflammatory cytokines [34]. Iron deficiency is a major cause of anaemia in this age group, and results from deficient dietary intake or extra-corporeal blood loss caused by chronic schistosomiasis and other parasitic infection such as hookworm, trichuriasis, or menstruation in girls. Other determinants of anaemia include micronutrient deficiencies such as vitamin A and B12, and folate; haemolysis caused by haemoglobinopathies or, if endemic, malaria; and anaemia of inflammation induced by pro-inflammatory cytokines released during chronic infections [33].

Co-infection of malaria with schistosomiasis has been related with under-recognized subtle morbidity which includes anaemia, splenomegaly, malnutrition, mortality, cognitive development, reproduction and physical work capacity [4]. From the study 16.4% of those infected with malaria are anaemic, 3.4% of those infected with schistosomiasis were anaemic while 5% of those co-infected were anaemic. It could be argued that co-infection does not take a toll on the packed cell volume, but the numbers are small for such a conclusion. In addition, the presence of *Schistosoma* co-infection during uncomplicated *P. falciparum* malaria unbalances the regulation of the associated inflammatory response [18].

The successful resolution of *Plasmodium* infection requires a coordinated succession from a T-helper cell type 1 (Th1) to a Th2 type response, and anything that upsets the timing or balance of this process can lead to chronic or severe infection [35]. The Th2-skewed immune profile and profound cellular hypo-responsiveness induced by chronic helminth infection might therefore be expected to affect the course of *Plasmodium* infection. Induction of an anti-inflammatory immunosuppressive network may prevent severe pathology in the later stages of malaria infection, with high levels of helminth-induced interleukin IL-10 acting to down-modulate the effects of interferon (IFN-γ) and tumor necrosis factor (TNF-á), thus reducing malaria pathology [15].

Anaemia is one of the most widespread and common health condition afflicting individuals living in the tropics, and in Africa, it contributes to 23% of nutrition-related disability-adjusted life years (WHO 2002). The consequences of anaemia are particularly

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severe for children and pregnant women. Chronic anaemia during childhood is associated with impairments in physical growth, cognition, and school performance, whereas severe anaemia accounts for up to one half of the malaria-attributable deaths in children younger than 5 years of age. Schistosomiasis also causes anaemia by chronic blood loss, as eggs penetrate the wall of the bowel (in intestinal schistosomiasis) and the urinary tract (in urinary schistosomiasis) [33]. Like in malaria, anaemia caused by schistosomiasis can additionally arise from destruction of red blood cells and/or dyserythropoiesis. Hepatosplenomegaly was observed in only one case in this study.

Conclusion

From this study, the effect of malaria co-infection with schistosomiasis on subtle morbidity is not clear. Although important information on cytokine immune response during co-infection has been obtained, it is expedient that factors such as varying sanitary standards, environmental conditions, personal hygiene and micro-geographical variations in exposure to the parasites vectors leading to prevalence of the disease should be curbed and an integrated control program be introduced.

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