

Anemia and Co-Infection of *P. falciparum* and Hookworm among School Children in Kinondoni District Dar es Salaam Tanzania

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Abstract: This was a cross-sectional study conducted in Kinondoni district from January to June 2015. The aim of the study was to determine the prevalence of co-infection of malaria and hookworm plus anemia in school-aged children. Stool and blood samples were collected from 332 children aged 6-14 years. Hemoglobin concentration (Hb) was determined by using portable HaemoCue. Giemsa-stained blood film was examined to detect malaria parasites while Kato Katz technique was used to detect hookworm ova. The Pearson's chi-square, Student's T-test, and Multivariate analysis were performed using SPSS version 20. The prevalence of malaria, hookworm, co-infection, and anemia were 13.5% 27.7% 7.5% and 22% respectively. The mean Hb was 10.51 g/dl (± 1.31). Mean Hb in co-infected children was low compared to those with a single infection. The prevalence of co-infection was high among 6-11 years. *Ascaris lumbricoides* was another parasite that was isolated in this study. This study showed that anaemia, malaria and hookworm co-infection are prevalent in school-aged children and an intervention to address the rate of malaria, hookworm, and anemia in the study area should be considered.

Keywords: *Plasmodium falciparum*, Hookworm, Co-infection, Anemia, School children

1. Introduction

More than 500 million people are infected with hookworms worldwide with majority of infection occurring in sub-Saharan Africa (SSA) [1]. Hookworm infection causes morbidity and about four million daily adjusted life-years (DALYs) lost annually [2]. In areas of stable malaria transmission, 32 million school aged children are at risk of co-infection with *P. falciparum* and soil transmitted helminth species, with the risk greatest for hookworm [3]. In Sub Saharan Africa 45 million school children are at risk of malaria and hookworm co-infection. [3]. Studies has reported that malaria and hookworm causes anemia that increases morbidity particularly in school children [4][5]. Anaemia interferes with school performance by impairing cognition attention and concentration in school children [6]. Anaemia was also reported to affect physical activities and growth [7] [8]. Anemia may also reduce capacity of red blood cells to carry oxygen to the tissue thus impair aerobic capacity in tissue performance. This may lead to cause reduced work capacity and increased heartbeat in slightest exercise. Also increased absences from school plus decreased school performance [9][10].

The risk of anemia may be aggravated by *Plasmodium falciparum* and hookworm co-infection as both parasites cause anemia by different mechanisms. *P. falciparum* causes anemia by increasing rate of destruction of red blood cells by removal of parasitized and non parasitized cells. Also, decrease the rate of red cells production in the bone marrow [11]. Hookworm cause anemia by direct intestinal blood loss [12]. Considering the different mechanisms by which malaria and hookworm reduce hemoglobin levels the occurrence of co-infection may bring in an additive impact. Presumably, this may reduce

anemia from mild level to moderate and severe forms with morbidity consequences in school children.

Studies on co infection of *P. falciparum* and hookworm have shown conflicting results. A community-based study conducted in Uganda showed that co-infection of hookworm and *Plasmodium* species causes a slight decrease in hemoglobin in school-age children [13]. A study in Ghana showed that anemia was higher among children infected with malaria alone than among children infected with hookworm alone or co-infected [14]. A study in Côte d'Ivoire found that children infected with hookworm and malaria co infection had lower haemoglobin compared to children infected with *P. falciparum* alone [15]. On the other hand a study on malaria and hookworm in Zanzibar revealed that hookworm was strongest associated with anemia compared to other infections [16]. Hookworm was responsible for 25% of all anemia, 35% of iron deficiency anemia, and 73% of all severe anemia cases in schoolchildren [16].

Data on the prevalence of *P. falciparum* and hookworm co-infection and morbidity associated with co-infection is limited in Tanzania. Most parasitic diseases are still studied individually or concomitant infection with soil-transmitted helminthes and *Schistosoma* specie [17][18][19]. In addition, existing control programme focus on a single infection despite occurrences of co-infection [20]. The magnitude and distribution of *P. falciparum* and hookworm co-infection and anemia in school-age children have not been studied in Tanzania. Therefore, this baseline cross-sectional study reports the magnitude of co-infection of *P. falciparum* and hookworm and their association with anemia and school attendance in schoolchildren in Kinondoni district, Dar es Salaam,

Tanzania. Knowledge of the magnitude of co-infections will guide in the development of intervention targeting co-infections in school aged children.

2. Methodology

2.1 Study area

The study was conducted in Kinondoni district, Dar es Salaam region. The district is located on the Northern part of Dar es Salaam. It has the longest coastline of the Indian Ocean. Like the rest of the areas in Dar es Salaam it is situated at Sea level with average temperatures of 30 degrees *Celcius* and very high humidity for the whole year. The main rainy season is from March to May with a second period from November to December, although occasional rain occurs at all times of the year. Average rainfall is 1200 to 2100 mm per year. Malaria transmission occurs throughout the year with peaks during the two rain seasons. Soil-transmitted helminth is endemic in the district. Mikocheni primary school was selected conveniently due to easier accessibility but parameters of study available in the school are comparable to other schools in Dar es Salaam and in the country at large.

2.2 Study design and sample size

This was a cross-section study that was conducted among children aged 6 to 14 years studying at Mikocheni Primary School from January to June 2015. The study population was all pupils at Mikocheni Primary School from standard one to standard seven. The sample size of 345 was determined using single population proportion formula. In this case the co-infection of malaria and hookworm was estimated at 25% [3]. Margin of error was set at 5%. To compensate for sampling design and attrition ten percent was added to the sample size as a contingency.

Children who had febrile illness at the time of reporting to school, children with a history of being treated for severe or complicated malaria in the past six months, child with a medical condition necessitating concurrent use of other drugs and children reported to have been admitted in hospital due to severe anemia or for blood transfusion in the previous month were excluded from the study.

2.3 Sampling technique

Systematic random sampling technique was used to select the study participants. List of children was obtained from the attendance register of each class. Sampling interval was calculated by dividing the total number of children by the sample size. A random number between one and the sampling interval was selected to be the starting point of the sample extraction. Subsequently, the study participants were selected by adding the sampling interval to the number corresponding to the previous number chosen on the list. The process was continued until the required number was obtained.

2.4 Social demographic data

Socio-demographic data and other information including school attendance in the previous month, reasons for absence from school in the previous month, history of treatment for malaria in the month prior to the investigation were collected using pre-tested structured questionnaire.

2.5 Identification and quantification of hookworm ova

Plastic containers and instructions on how to collect stool sample were provided to the children the day before screening started. Stool containers were then collected at the school in the morning and labelled with identification numbers. Stool samples were then taken to Hubert Kairuki University laboratory and examined within one hour of preparation to avoid missing hookworm ova. Kato-Katz cellophane thick smears was prepared from each specimen in duplicates using 41.7 mg templates [21]. The mean number of eggs from each Kato Katz thick smear was multiplied by 24 in order to express infection intensities as the number of eggs per gram (epg) of feces [22]. Infection intensity of hookworms was classified as light (< 1000 epg), moderate infection (1000-3999 epg) and heavy infection (> 4000 epg) [22].

2.6 Blood test for identification of malaria parasites and hemoglobin

The sample for haemoglobin and malaria parasites was obtained from capillary blood on the fingertip of each child. The finger tip was cleaned with 70% ethanol before pricking the finger using disposable sterile blood lancets. All safety procedures were followed during blood collection. Thick blood films were created, air-dried, stained with Giemsa stain, and observed under the microscope for identification and quantification of malaria parasites. Malaria parasites were counted against 200 leukocytes, and counts were expressed as the number of parasites per micro litre of blood, assuming an average leukocyte count of 8,000 cells/ μ l blood [22]. For a quality control measure, 10% of randomly selected smears were re-examined by another technician who was blinded of the previous results. Haemoglobin concentration was assessed using a portable HemoCue Hb 301 device (HemoCue AB; Ångelholm, Sweden). Children with hemoglobin (Hb) levels lower than 11.5 g/dl for age ranges from 5–11 and 12 g/dl for age ranges 12–14 years old were considered anemic [23]. Mild anemia was defined as Hb concentration of 11–11.9 g/dl for 12–14 years and Hb concentration of 11–11.4 g/dl for 5–11 years. Moderate anemia was defined as Hb concentration between 8–10.9 g/dl and severe anemia was defined as Hb concentration lower than 8 g/dl [23]

2.7 Data management and analysis plan

Data entry, storage, and analysis were done using SPSS version 20 for windows. Raw data were entered in double to minimize errors during data entry. Student t-test and Analysis of variance (ANOVA) were used to compare means for continuous variables. Proportions were

compared by using the chi-square test, and logistic regression analyses were done to obtain the predictor of anaemia. Statistical significance was set at $p \leq 0.05$ and 95 % CI was used.

2.8 Ethical considerations

Ethical clearance was obtained from the Institutional Research Ethics Committee (IRIC) of the Hubert Kairuki Memorial University before commencing of the study. Prior to conducting the study, meetings were held with parents/guardians and teachers to explain the aims and procedures to be used to collect data. Informed written consents were obtained from children's parents or guardians. In addition assent was obtained from the children. Participants who were diagnosed with parasites were treated according to the National guidelines. Children with severe anemia were referred to the nearest health facility for medical management.

3. Results and Discussion

A total of 332 out of 345 school children were recruited in the study after receiving the signed informed consent from their parents. However, 13 children did not turn up for blood and stool collection and were excluded from the study. The response rate was 96%. The mean age of the study population was 10.13 ± 2.5 standard deviations (SD). Females were 184(55.4%) and children aged 7-11 years of age were 148 (44.6%). Children were from Kawe 28(8.43%), Mikocheni 117(35.25%), Makumbusho 60(18.07%), Msasani 72(21.69%) and Mwananyamala 55(16.56%).

3.1 Prevalence and intensity of malaria by age, gender, and residence

Out of 332 forty-five (45), children were positive for malaria, resulting in a prevalence of 13.6 % (95 % CI: 12.19-15.01). The prevalence of malaria was higher among females (23/184[12.5.0%]) than males (22/148 [14.9 %]) however, the difference was not significant. ($\chi^2 = 0.392$, $p = 0.532$). The prevalence of malaria was higher in children aged 6-11 years (40/206 [19.4 %]) compared to children aged 12 to 14 years of age (5/126 [4.0 %]). The difference was significant ($\chi^2 = 15.92$, $p = 0.000$).

Children from Mikocheni area had a higher prevalence of malaria 4.8 % (16/332) followed by Msasani 3.6% (12/332). Prevalence of malaria in Makumbusho and Mwananyamala was 1.8% (6 /332) respectively. The lowest prevalence of malaria was recorded at Kawe 1.5% (5/332). Area of residence was not associated with malaria prevalence $\chi^2 = 5.10$, $p = 0.27$

Mean malaria density was low 113.33 ± 66.14 SD and the range was 40-250 parasite per μ l. Mean parasite density was higher in the age group 6-11 years compared to age group 12-14 years. However, the difference was not significant $t = -1.187$ $p = 0.242$. There was no significant difference between malaria intensity and gender ($t = 0.483$, $p = 0.631$)

3.2 Prevalence and intensity of hookworm

Among the 332 children, 92 were positive for hookworm parasites, resulting in a prevalence of 27.7 % CI = (24.45-30.62). Hookworm was more prevalent in children aged between 12 to 14 years (47/126 [37.3 %]) compared to children 6 to 11 years of age (45/206 [21.8 %]). The difference was significant ($\chi^2 = 9.24$, $p = 0.002$). The prevalence of infection was higher in females (48/184 [26.1 %]) than in males (44/148 [29.7 %]), however, the difference was not statistically significant ($\chi^2 = 0.543$, $p = 0.461$).

Hookworm intensity was light with mean intensity 1110.74. Mean hookworm intensity was high in the age group 6-11 years (1421) than in age group 12-14 (834), the difference was significant $t = 3.184$, $p = 0.002$. Females had higher mean intensity (675.51 ± 272), compared to males (579 ± 266), however, the difference was not significant $t = -1.917$, $p = 0.058$.

3.3 Hookworm and *P. falciparum* co-infection

Out of 332 children, 25 were co-infected, resulting in a prevalence of co-infection of 7.5% (95 % CI: (7.02-8.58). Co-infection was more prevalent in the age group 12-14 (14/126[11.1%]) compared to the age group 6 to 11 years (12/206[5.8%]). The difference was significant ($\chi^2 = 21.99$, $p = 0.000$) (Table 1). The prevalence of co-infection was higher in females (17/184 [9.2%]) than in males (9/148[6.1%]). However, the difference was not significant ($\chi^2 = 2.653$, $p = 0.448$)

Table 1: Prevalence of *P. falciparum* hookworm and co- infection by Age and Gender

Age group	No. Examined	PF No. %	p-value	HW No. %	p-value	Co infection MP+HW No / %	p-value
6-11	206	40 (88.9)		45(21.8)		20(9.71)	
12-14	126	5 (11.1)	0.0	47(37.3)	0.00	5 (3.96)	0.05
Total	332	45 (13.5)		92(27.7)		25 (7.5)	
Gender							
Male	148	22(14.8)		44 (29.7)		13 (8.78)	
Female	184	23 (12.5)	0.5	48 (26.1)	0.46	12 (6.52)	0.43
Total	332	45 (13.5)		92(27.7)		25 (7.5)	

Key: PF = *P. falciparum* HW = Hookworm

3.5 Prevalence of anemia

In this study, 73 of the 332 participants were anemic resulting in a prevalence of anemia of 22% (CI =19.68-24.31). The prevalence rates of mild, moderate, and severe anemia were 25%, 12.0% and 2.7% respectively. Anemia was more prevalent in children aged 6-11 [58.9% (43/73)]

compared to children aged 12-14 [41.1% (30/73)]. However, the difference was not significant $\chi^2=0.393$ $p=0.585$. The prevalence of anemia was higher in females [54.5% (40/73)] compared to males [45.2% (33/73)]. The difference was not significant $\chi^2 = 0.015$, $p=1.00$. Hookworm and co-infection were associated with anemia but malaria was not associated with anaemia (Table 2).

Table 2: Association of anemia hookworm malaria and co infection

Type of infection	No.	Anemia				X ²	p-value
		Absent		Present			
		No.	%	No.	%		
Hookworm	92	58	64.1	34	36.9	131.76	0.000
Malaria	45	17	37.8	28	62.2	7.56	0.006
Co-infected	25	14	56.0	11	44.0	18.23	0.000

The prevalence of severe anemia was 8.7% in children who were infected with hookworm, 4.4% in children who were infected with malaria and 22.2% for children who

were co-infected. Level of anemia was associated with hookworm and co- infection. Malaria was not associated with level of infection (Table 3).

Table 3: Association of different levels of anemia with hookworm *P. falciparum* and co infection

Type of infection	No infected.	Type of anemia						Fisher exact test	p value
		Mild	%	Moderate	%	Severe	%		
Hookworm	92	16	17.1	34	37.0	8	8.7	118.4	0.00
Malaria	45	7	15.9	8	17.8	2	4.4	8.70	0.24
Co infection	25	5	20.0	7	28.0	2	22.2	17.28	0.00

In this study the mean hemoglobin (\pm SD) was 11.41(\pm 1.33g/dl). (Range7.0-13.0). There was no significant difference observed in mean hemoglobin between those who had malaria and those who were not infected with malaria. Children with hookworm infection

had low mean hemoglobin compared to children who were free from hookworm infection. The difference was significant (Table 4) Children who were co-infected had low mean hemoglobin compared to children who were not co-infected. The difference was significant (Table 4)

Table 4: Comparison of HB (g/dl) among the study population

Type of infection		Mean Hb \pm SD	p - value	95%CI
Malaria	Negative	11.5 \pm 1.30	0.128	-0.09-.074
	Positive	11.1 \pm 1.46		
Hookworm	Negative	11.9 \pm 0.68	0.000	1.27-2.03
	Positive	10.3 \pm 1.78		
Co-infection of <i>P. falciparum</i> and hookworm	Not Co-infected	11.5 \pm 1.28	0.004	0.25-1.32
	Co infected	10.6 \pm 1.6		

3.6 Malaria intensity and hemoglobin concentration

Low hemoglobin concentration was recorded in children with higher density of malaria parasites >200 parasites per micro litre of blood. Children with less than 200 parasites

per micro litre had higher hemoglobin level compared to children with more than 200 parasites per micro litre of blood. The difference was significant. $F=62.258(1)$, $p=0.000$ (Table 5)

Table 5: Association between malaria parasite density and mean hemoglobin

Malaria parasites	N	Mean Hb	Std. Deviation	95% Confidence Interval for Mean	
<200parasites per micro liter	27	12.0	0.19	11.9	12.1
>200 parasites per micro liter	18	9.7	1.47	9.0	10.5
Total	45	11.1	1.45	10.6	11.5

There was statistically significant difference between hookworm intensity and mean hemoglobin as determined by one-way ANOVA. Hemoglobin concentration

decreases with increasing hookworm intensity $F=78.87$, $p = 0.000$ (Table 6).

Table 6: Association between hookworm intensity and mean hemoglobin

Hookworm intensity	N	Mean Hb	Std. Deviation	95% Confidence Interval for Mean	
Light infection (< 1000 epg)	57	11.3	1.2	10.97	11.58
Moderate infection (1000-3999 epg)	28	8.8	1.0	8.42	9.21
Heavy infection (> 4000 epg)	7	7.1	0.4	6.79	7.49
Total	92	10.2	1.8	9.84	10.58

3.7 Attendance and parasite prevalence

Out of 332 children 182 (54.8%) reported to have fallen sick in the previous month and missed school due to different illness. The illness included stomach ache 20(10.9%) fever and headache 100 (30.1%) chest pain and cough 62 (18.7%). Children who were sick received treatment in different health facilities including hospital 98 (29.5%) and health centre 34(10.2%). About 13(3.9%) brought medication from pharmacy however some of the

children were not treated because their illness resolved after taking painkillers. Only few children were able to mention medication that they were treated with.

Among children reported to have missed school in the previous month 48(26.4%) were anemic, 20(11.0%) had malaria 55(30.2%) had hookworm and 10(5.5%) were co-infected with hookworm and *P. falciparum*. Missing school in the previous month was associated with anaemia (Table 7).

Table 7: Association between missing school in the previous month and prevalence of parasites and anaemia

	No.	Absence in the previous month					
		Yes		No		χ^2	P value
		No. . . (%)	No. (%)	No. (%)	No. (%)		
Anemia	73	48(26.4)	25(34.2)	4.51	0.03		
Malaria	45	20(11.0)	25(16.2)	2.26	0.14		
Hookworm	92	55(30.2)	37(24.7)	1.26	0.27		
Co-infected	25	10(5.5)	15(4.5)	2.39	0.14		
Total	235	182(54.8)	150(45.1)				

3.8 Predictors of anemia

Multivariate regression analysis revealed that hookworm was significantly associated with anemia. For every unit

increase in hookworm infection the Hb level decreased by 3% ($P < 0.0001$). Malaria and co- infection was not associated with Hb (Table 8).

Table 8: Multivariate logistic regressions of selected variables associated with anemia among school-age children in Kinondoni district

Predictors of anaemia	B	Wald	p value	Odds ratio	95% C. I	
					Lower	Upper
Malaria	-.581	.577	.447	.559	.125	2.503
Hookworm	-3.882	76.295	.000	.021	.009	.049
Co-infected	-1.415	2.465	.116	.243	.042	1.421
Age group	.798	3.694	.055	2.220	.984	5.007

In this study, the prevalence of *P. falciparum* and hookworm co-infection among study participants was 7.5% which confirms that co-infection of *P. falciparum* and hookworm is prevalent in school children in Kinondoni district. This result conforms to the prevalence of co-infection reported in school children in other areas of Tanzania which showed that school children carry a high burden of co-infection. [18][17]. The higher prevalence of hookworm and *P. falciparum* co-infections observed in Kinondoni reflect the favorable climate and similar co-distribution of these two species[3]. Stable and perennial transmission of malaria occurs in the warm humid coastal regions [24] where hookworm are also prevalent. The prevalence of co-infections observed in Kinondoni differs from those reported in other areas of Tanzania. Our results show lower prevalence of hookworm and *Plasmodium* co-infections than that of Mvomero [25] but higher than that of Magu [17] and Bagamoyo [18]. Factors such as methods of detection and exposure as well as coverage of bed net and mass drug administration interventions might be the reason for these differences in the prevalence.

Studies have reported that even with above 80% coverage of Long lasting Treated Bed Nets (LLINs) there are still pockets of *Plasmodium* infection that remains in some areas[18]. Behaviour factors such as outdoor activities use of LLINs and sanitation may also contribute to difference in prevalence. In addition, easy access to health care and effective treatment may also cause the differences in prevalence observed in these areas.

The prevalence of co infection in this study was low compared to the prevalence of co-infection reported in other countries [15][26][27][13]. Regular de-worming and free bed net campaign run by the Ministry of health in Tanzania may account for the low prevalence of hookworm and *P. falciparum* co-infection in Tanzania.

The prevalence of malaria was 13.5%. This confirms previous reports that malaria is low in this area due to the presence of malaria control campaign that has been going on in the country[18][28]. There was a significant association between the prevalence of malaria and age

which is in line with several other studies done elsewhere [18][29][30]. The mean parasite count for *Plasmodium falciparum* was higher in age group 6-11 compared to age group 12-14. This could be explained by the development of malaria specific immunity which increase with increasing age [31][32]. However, some studies have reported a change of *Plasmodium* infection towards older age group where malaria transmission tends to decline and immunity acquisition is hence delayed [29][33]. For that reason, interventions to control malaria should consider the older age group in the prevention of malaria[34].

Our study recorded an overall prevalence of 27.7% for hookworm infections. This was higher compared to reported prevalence in Mbeya Tanzania [35]. The higher prevalence of hookworm in this study may be influenced by factors such as behavioural, sanitation and soil types. Poor sanitary conditions prevail in most of the slum areas of Dar es Salaam due to unplanned settlements people use unhygienic methods to empty their latrines. As a result, populations are regularly exposed to fecal sludge in overly full pits during use and frequent releases into local communities[36]. The fact that most of the children remove their shoes and play barefooted might have exposed them to infective stage of hookworm larvae. Therefore de-worming campaign run by the Ministry of health should go hand in hand with sanitation and health education to students and community. Soil type has been shown to influence hookworm infection [37]. High sand content of the soil favour the development of hookworm larvae, because sand soils tend to absorb water and is well aerated. This condition exists in Kinondoni district and likely influenced the high hookworm burden observed.

Hookworm infection was more prevalent in older children aged compared to young children which conforms to the studies which were done elsewhere [15] This could be attributed to differences in exposure level in children as they grow. Normally highest helminth infection tend to occur among older children and adolescent [38]. Another intestinal worm isolated was *Ascaris lumbricoides*. However the predominant parasite was hookworm and this is in line with other studies conducted in coastal areas [18][16][39].

3.9 Anaemia

About 22% of the children were diagnosed with anaemia, mostly being mild to moderate. Hookworm was the only predictor of anaemia in multivariate analysis. Co-infection was not a predictor of anaemia. This finding conforms previous studies conducted in Côte d'Ivoire [15] and Uganda [13] indicating complex nature of interaction between *Plasmodium falciparum* and hookworm which might be of advantage to the host. The decreasing trend in anaemia prevalence with increasing age recorded in this study is similar to that of other reports [40][15]. This might be due to the acquisition of malaria immunity with increasing age. Therefore, younger age group carrying higher malaria parasite densities and high intensity of hookworm are prone to anaemia. While malaria parasite density was not identified as a predictor of anaemia in

multivariate regression model, a significant different was observed between malaria prevalence and anaemia.

4. Conclusion

Anaemia, malaria and hookworm co-infection are prevalent in school-aged children. Intervention to address the rate of malaria, hookworm, and anemia in the study area is needed to prevent adverse consequences in children's learning.

5. Future Scope

Future study should consider other factors which may also contribute to anaemia like human immunodeficiency virus (HIV) use of antiretroviral drugs and antibiotics genetic disorders, such as sickle cell disease and nutritional deficiency such vitamin B12, A, and folic acid.

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