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Renal related disorders in concomitant *Schistosoma haematobium*–*Plasmodium falciparum* infection among children in a rural community of Nigeria

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Summary Schistosomiasis and malaria are two common parasitic diseases that are co-endemic in resource-poor communities of sub-Saharan Africa. This study aims to assess the effects of single and concomitant *Plasmodium falciparum* and *Schistosoma haematobium* infections on two indicators of renal injury in school children in a rural community of Nigeria. A cross-sectional epidemiological survey was carried out on a total of 173 schoolchildren between ages 6 and 18 years (mean age 11.4 ± 2.6 years). Urine and blood samples were collected by standard methods for concurrent microscopic diagnosis of *S. haematobium* and *P. falciparum* infections. Urinary blood (hematuria) and protein were determined using a urinalysis dipstick. The prevalence of single infections was 75.1% and 78.2% for *S. haematobium* and *P. falciparum*, respectively. A total of 57.1% individuals were infected with the two parasites. The prevalence of hematuria was significantly higher in the co-infection status (63.8%) than in single *S. haematobium* (52.2%) and *P. falciparum* (43.7%) infection statuses ($p = 0.04$), while no significant variation was recorded in proteinuria in the three

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infection statuses ($p = 0.53$). The proportion of children with renal injury associated with the co-infection of these parasites is very high, particularly in young children, who seem to have a higher prevalence of hematuria.

Introduction

Multiple parasitic infections are common in sub-Saharan Africa countries with concurrent infection patterns influencing the pathogenesis of each parasite [1]. Antagonistic or synergistic interactions of parasites in animal models have been suggested to show similar phenomena in humans [2]. In Africa, malaria and schistosomiasis are the first and second most important parasitic diseases with public health implications, respectively [3,4], with cases of concomitant occurrence in many epidemiological studies conducted in the region [2,5,6].

There have been convincing reports about the additive morbidity effects of co-infection of *Plasmodium falciparum* and *Schistosoma haematobium* compared with the single infection of each parasite. Increased anemia and organomegaly in concurrent infections of the parasites have been documented [5,7]. In addition, a recent study examined the effect of concomitant infection of the two parasites on hematologic profiles [6].

Operational research in sub-Saharan Africa has widely adopted the use of hematuria and proteinuria as diagnostic indicators of urogenital schistosomiasis being the morbidity indicators of infection by *S. haematobium* [8—10]. Likewise, in malaria parasite infections, the two urinalysis parameters have been assessed, and it can be concluded that while *P. falciparum* infection is closely associated with proteinuria, its association with hematuria is inconclusive [11,12]. At the time of this report, no study has unraveled the effects of co-existence of *P. falciparum* and *S. haematobium* on hematuria and proteinuria in endemic areas of sub-Saharan Africa. Therefore, this study assessed the synergistic effects of the concomitant occurrence of the two parasites on abnormal urinary blood and protein excretion among school children in a low resource rural community in Nigeria.

Methods

Study area and data sources

The study was conducted in Ijaka-Isale village in the Yewa North Local Government Area (LGA), Ogun State, Nigeria, an adjacent, neighboring community to Ijaka-Oke in the same LGA. The village shares a similar sociodemographic and socioeconomic status with Ijaka-Oke [6]. The community population size is approximately 1000 and shares similar sociocultural practices, such as water storage in open earthen vessels for mosquito breeding [6], with Ijaka-Oke. Yewa River flows downstream from Ijaka-Oke to the community.

Study design

The study was conducted on school pupils in Ijaka-Isale. The population structure of the subjects was similar to that of Ijaka-Oke with ages ranging from 6—18 years (mean age 11.4 ± 2.6 years). The cross-sectional and descriptive study adopted similar sampling procedures as reported by Morenikeji et al. [6]. Using 50.0% prevalence of schistosomiasis and malaria, 0.8 precision, and 90.0% statistical power, the minimum sample size was 150. A non-randomized sampling procedure was used to recruit the subjects.

Inclusion and exclusion criteria

All children who had lived in the area for at least one year and whose parents or guardians gave written informed consent were included in the study. Those showing evidence of acute or chronic illness were excluded from the study. Visitors were also not included in the study. Children positive for *S. haematobium* were administered Praziquantel at a dose of 40 mg/kg of body weight, while children who tested positive for malaria parasites were

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referred to the health clinic centers, where they were treated according to current WHO standards.

Ethical considerations

The Ogun State Ministry of Health and the University of Ibadan/University College Hospital Institutional Ethical Review Board (UI/EC/13/0091) granted approval for this study. The subjects' parents and guardians also provided written informed consent.

Parasitological diagnoses

The subjects were provided with dry, clean, screw-capped 15-mL universal bottles. Mid-stream urine collected under proper guidance and monitoring between the hours of 10 am to 2 pm was visually observed for gross hematuria and screened for trace blood occurrence (microhematuria) and urinary proteinuria using Combi Screen 10SL (Analyticon (R) GMBH D-35 104, Litchenfel Germany). The urine samples were transported to the laboratory for further analysis. Ten milliliters (10 mL) of each sample was measured and centrifuged at 4000 rpm for 4 min. Concentrated eggs in the sediment were viewed under ×10 microscope magnification following the discarding of the supernatant. A terminally spined egg with an elliptical shape was considered to be *S. haematobium* [13].

Thick blood smears for the determination of malaria parasite positive blood samples and thin blood smears to determine malaria parasite species were prepared from the blood samples collected by venipuncture and stained with 10% Giemsa. Species were identified under a light microscope at a high magnification (×100 under oil immersion) by counting the number of parasites per 200 white blood cells (WBC). All smears present for either ring forms or gametocytes were considered to be positive for plasmodial infection. The control group was selected from the children who tested negative for *P. falciparum* and *S. haematobium* infections.

Data analysis

The analysis of the data was performed by GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA 92037, USA). The statistical significance of differences in the prevalence of single and co-infection status was determined via Chi-square analysis. Multivariate logistic regression analysis was used to predict the extent of the association between the variables and disease occurrence. $p < 0.05$ was considered statistically significant.

Results

A total of 182 children were recruited, 173 of which were screened for *S. haematobium* infection and 147 who were screened for malaria parasites and co-infection of the two parasites (Table 1). *Plasmodium falciparum* was the only species of *Plasmodium* diagnosed in the screened subjects. The prevalence of single infection was 75.1 and 78.2% for *S. haematobium* and *P. falciparum*, respectively. The prevalence of urogenital schistosomiasis varied significantly ($p = 0.018$) with the ages of the children, with the highest prevalence ($p = 87.1\%$, Odd ratio (OR) = 3.1) and the lowest prevalence ($p = 60.0\%$, OR = 0.5) recorded in ages 6—8 and ≥14 years, respectively (Table 2). The prevalence of *S. haematobium* was, however, not gender dependent ($p = 0.975$) (Table 2). There was no association between the prevalence of malaria and the ages of the children ($p = 0.507$). The same was observed in male ($p = 73.8\%$, OR = 0.6) and female subjects ($p = 83.6\%$, OR = 1.8) ($p = 0.150$) (Table 3).

A total of 57.1% of individuals were infected with the two parasites, with those in the lowest age group (6—9 years) being the most predisposed to the infections ($p = 62.3\%$, OR = 1.3). Gender prevalence pattern of *S. haematobium* and malaria parasite co-infection showed no significant difference ($p = 0.214$) (Table 4). The prevalence of hematuria

Table 1 Characteristics of the study population.

Categories		No. recruited	No. screened		
			Schistosomiasis (%)	Malaria (%)	Co-infection (%)
Age (years)	6—9	65	62 (35.8)	53 (36.1)	53 (36.1)
	10—13	99	96 (55.5)	81 (55.1)	81 (55.1)
	≥14	18	15 (8.7)	13 (8.8)	13 (8.8)
Gender	Male	98	94 (54.3)	80 (54.4)	80 (54.4)
	Female	84	79 (45.7)	67 (45.6)	67 (45.6)
	Total	182	173	147	147

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Table 2 Age and sex related urogenital schistosomiasis infection status among school-aged children in Ijaka Isale.

Categories	Variables	No. examined	No. infected	Prevalence (%)	OR (95% CI)	*p*-value
Age (years)	6–9	62	54	87.1	3.1 (1.3–7.2)	0.018
	10–13	96	67	69.8	1.9 (0.9–4.0)	
	≥14	15	9	60.0	0.5 (0.2–1.4)	
Gender	Male	94	70	74.5	0.9 (0.5–1.8)	0.975
	Female	79	60	75.9	1.1 (0.5–2.2)	
	Total	173	130	75.1		

was significantly higher in the co-infection status ($p = 63.8\%$, OR = 2.2) than in single *S. haematobium* ($p = 52.2\%$, OR = 1.3) and *P. falciparum* ($p = 43.7\%$, OR = 0.8) infection statuses ($p = 0.04$). Although no significant variation was recorded in proteinuria in the three infection statuses ($p = 0.53$), individuals co-infected with the two parasites appeared to have a higher risk of abnormal urinary protein ($p = 57.4\%$, OR = 1.5) (Table 5). The mean hematuria (161.7 ± 107.6 ery/μL urine) in the co-infection status was significantly higher than in single *S. haematobium* (150 ± 109.7 ery/μL urine) and *P. falciparum* (159.7 ± 108.8 ery/μL urine) infection statuses ($p = 0.04$). There was, however, no

Table 3 Age and gender related malarial parasites status among children in Ijaka Isale.

Categories	Variables	No. examined	No. infected	Prevalence (%)	OR (95%CI)	*p*-value
Age (years)	6–9	53	40	75.5	0.8 (0.3–1.7)	0.507
	10–13	81	66	81.5	1.5 (0.7–3.4)	
	≥14	13	9	69.2	0.6 (0.2–2.1)	
Gender	Male	80	59	73.8	0.6 (0.2–2.0)	0.150
	Female	67	56	83.6	1.8 (0.8–4.1)	
	Total	147	115	78.2		

Table 4 Concomitant *S. haematobium* and malarial parasites infections in school-aged children in Ijaka Isale.

Categories	Variables	No. examined	No. infected	Prevalence (%)	OR (95%CI)	*p*-value
Age (years)	6–9	53	33	62.3	1.3 (0.7–2.8)	0.524
	10–13	81	45	55.6	0.9 (0.4–1.7)	
	≥14	13	6	46.2	0.6 (0.2–1.9)	
Gender	Male	80	42	52.5	0.7 (0.3–1.3)	0.214
	Female	67	42	62.7	1.5 (0.8–2.9)	
	Total	147	84	57.1		

Table 5 Hematuria and proteinuria profiles in single and coinfection statuses.

Indicators	Status	Control ($n = 97$)	Infection status			
			Malaria ($n = 71$)	Schistosoma ($n = 67$)	Co-infection ($n = 47$)	*p*-value
Hematuria (ery/μL)	Positive	39 (40.2)	31 (43.7)	35 (52.2)	30 (63.8)	0.044
	Negative	58 (59.8)	40 (56.3)	32 (47.8)	17 (36.2)	
	OR (95%CI)	0.6 (0.4–1.0)	0.8 (0.5–1.4)	1.3 (0.7–2.2)	2.2 (1.1–4.2)	
Proteinuria (mg/dL)	Positive	43 (44.3)	35 (49.3)	33 (49.3)	27 (57.4)	0.534
	Negative	54 (55.7)	36 (50.7)	34 (50.7)	20 (42.6)	
	OR (95%CI)	0.8 (0.5–1.3)	1.0 (0.6–1.7)	1.0 (0.6–1.8)	1.5 (0.8–2.8)	

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Table 6 Mean values of urine biochemical of school aged children in Ijaka-isale.

Indicators	Mean ± S.D.				*p*-value
	Control	Schistosomiasis	Malaria	Co-infection	
Hematuria (ery/μL)	118.0 ± 114.8	150 ± 109.7	159.7 ± 108.8	161.7 ± 107.6	0.041
Proteinuria (mg/dL)	15.6 ± 22.5	20.5 ± 29.2	20.6 ± 29.4	26.1 ± 35.1	0.438

significant difference in the mean values of proteinuria in the different infection categories ($p = 0.44$) (Table 6).

Discussion

This study showed very high endemicity of schistosomiasis and malaria in the area. The prevalence of *S. haematobium* (75.1%) recorded in this study is significantly higher than previous studies conducted in the same local government area among different population strata. For example, a 9.8% prevalence level was reported in preschool children [14], 57.1% in school children [10], and 20.8% in gestational women [15]. Likewise, the malaria prevalence in our study is higher than in other reports in Ogun State, Nigeria [16,17].

More importantly, *S. haematobium* and *P. falciparum* are more endemic in the study area than in a neighboring village (Ijaka Oke), according to a recent report [6]. In addition to poor socio-economic status, lack of potable water, sociocultural practices and the presence of snail intermediate hosts and mosquito vectors, the topography of the area may play a major role in the significant variations in the prevalence of these infections. The topographic terrain of the two communities is such that Ijaka Oke is more or less a highland with the downward flow of the Yewa River to a lowland Ijaka Isale village. This could have a significant impact on the malaria and *Schistosoma* vector distributions, which are likely to be more concentrated in areas where there is a slow water current. The influence of topography on *Schistosoma* and malaria parasite transmission had been emphasized [18,19].

The highest prevalence of concomitant infection (57.1%) of the two parasites ever recorded in Nigeria and other developing countries was observed in this study. In Ethiopia, an 18.4% concomitant infection prevalence level was reported [20], while countries like Zambia [21] and Tanzania [22] have recorded 10.7% and 22.6%, respectively. Our observations on the lack of association between the co-infection of the parasites with the age and gender of the children are consistent with other studies [6,20,21]. This suggests the equal predisposition of children of all ages and genders to concomitant *S. haematobium* and *P. falciparum* infections.

The prevalence of hematuria (49.3%) in *P. falciparum*-infected subjects in our study is similar to the 45.0% prevalence recorded in a previous report [11]. Similar observations were recorded for proteinuria, for which our recorded prevalence of 49.3% corroborated the 45.9% observed value in a previous study [12]. This study is the first of its kind to determine the effects of malaria and *Schistosoma* parasites' concomitant occurrence on hematuria and proteinuria. While many studies have related *P. falciparum* to proteinuria [12,23,24], there is a paucity of data on the effects of malaria on urinary hematuria [11,25]. This study suggested that co-infection of the two parasites could exacerbate renal disorders, as evidenced by a higher risk of hematuria and proteinuria compared with the single infection statuses. While malaria parasite-induced kidney complications may present glomerular endothelial activation and damage, as well as tubulointerstitial damage, resulting in impaired permeability and tubular reabsorption [12]; conditions that ultimately result in excessive protein excretion and hematuria [26,27], *Schistosoma*-associated glomeruli pathology, which results in red blood cells and protein leakage, are not uncommon [10]. Thus, co-infection of the two parasites will contribute more to renal related disorders. A limitation in this study is the use of single urine and blood sample for schistosomiasis and malaria diagnosis. This could lead to the under-presentation of the true infection status. Additionally, studies on bionomics of the snail intermediate hosts and mosquito vectors were not carried out to further support the effects of topography on the distributions of schistosomiasis and malaria in the area.

Conclusion

This study shows a very high endemicity of schistosomiasis and malaria in the study area. It further affirms the claim of a high coexistence of the causal

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organisms of the diseases. The co-infection of *P. falciparum* and *S. haematobium* contributed more to hematuria and proteinuria in children in resource-poor communities endemic for the two parasites. Integrated control measures, including snail and mosquito control, intermittent malaria treatment, deworming, provision of good water supply and education, should be adopted. These will not only reduce the prevalence to a significantly lower level but will also abate the burdens and co-morbidities associated with the co-infection of the parasites.

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Competing interests

None declared.

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References

[1] Cox FEG. Concomitant infections, parasites and immune responses. Parasitology 2001;122:S23—8.
[2] Briand V, Watier L, Hesran J-Y, Garcia A, Cot M. Co-infection with *Plasmodium falciparum* and *Schistosoma haematobium*: protective effect of schistosomiasis on malaria in Senegalese children. Am J Trop Med Hyg 2005;72:702—7.
[3] Crompton DWT, Montressor A, Neisheim MC, Savioli L. Controlling disease due to helminth infection. Geneva: World Health Organization; 2003.
[4] Snow RW, Guerra CA, Noor AM, Myint HY, Hay SI. The global distribution of clinical episodes of *Plasmodium falciparum* malaria. Nature 2005;434:214—7.
[5] Deribew K, Tekeste Z, Petros B. Urinary schistosomiasis and malaria associated anemia in Ethiopia. Asian Pac J Trop Biomed 2013;3:307—10, http://dx.doi.org/10.1016/S2221-1691(13)60068-4.
[6] Morenikeji OA, Atanda OS, Eleng IE, Salawu OT. *Schistosoma haematobium* and *Plasmodium falciparum* single and concomitant infections; any association with hematologic abnormalities? Pediatr Infect Dis 2014, http://dx.doi.org/10.1016/j.pid.2014.11.001.
[7] Nacher M, Singhasivanon P, Silachamroon U, Treeprasertsu S, Krudsood S, Gay F, et al. Association of helminth infections with increased gametocyte carriage during mild falciparum malaria in Thailand. Am J Trop Med Hyg 2001;65:644—7.
[8] French MD, Rollinson D, Basáñez MG, Mgeni AF, Khamis IS, Stothard JR. School-based control of urogenital schistosomiasis on Zanzibar, Tanzania: monitoring micro-hematuria with reagent strips as a rapid urological assessment. J Pediatr Urol 2007;3:364—8, http://dx.doi.org/10.1016/j.jpurol.2007.01.198.
[9] Ugbomoiko US, Dalumo V, Ariza L, Bezerra FSM, Heukelbach J. A simple approach improving the performance of urine reagent strips for rapid diagnosis of urogenital schistosomiasis in Nigerian schoolchildren. Mem do Inst Oswald Cruz 2009;104:456—61.
[10] Morenikeji O, Junaid Q, Omoregie C, Hassan A, Nwuba R, Anumudu C, et al. A cross-sectional study on urogenital schistosomiasis in children; hematuria and proteinuria as diagnostic indicators in an endemic rural area of Nigeria. Afr Health Sci 2014;14:390—6.
[11] Karoum AEGO, Mohammed BA. Urine analysis in malaria in Kassala town, Eastern Sudan. Saudi J Kidney Dis Transpl 2000;11:208—10.
[12] Lubis M, Ramayati RR, Ramayani OR, Siregar RS, Supriatmo. Proteinuria and malaria parasite counts in children. Paediatr Indones 2013;53:295—8.
[13] Loker ES. A comparative study of the life-histories of mammalian schistosomes. Parasitology 1983;87:369—73.
[14] Salawu OT, Odaibo AB. Urogenital schistosomiasis and urological assessment of hematuria in preschool-aged children in rural communities of Nigeria. J Pediatr Urol 2014;10:88—93.
[15] Salawu OT, Odaibo AB. Schistosomiasis among pregnant women in rural communities in Nigeria. Int J Gynecol Obstet 2013;122:1—4.
[16] Jonathan KA, Agbolade OM. *Plasmodium* and *Loa loa* infections among secondary school teachers and students, and ante-natal out-patients in Ijebu North area, Southwestern Nigeria. Int Res J Microbiol 2012;3:253—7.
[17] Olasunkanmi OI, Akingbade OA, Akinjinmi AA, Okerentugba PO, Onajobi IB, Okonko IO. Prevalence of malaria *Plasmodium* among children in Abeokuta, Nigeria. Acad Arena 2013;5:44—7.
[18] Leonardo LR, Rivera PT, Crisostomo BA, Sarol JN, Bantayan NC, Tiu WU, et al. A study of the environmental determinants of malaria and schistosomiasis in the Philippines using Remote Sensing and Geographic Information Systems. Parassitologia 2005;47:105—14.
[19] Githeko AK, Ayisi JM, Odada PK, Atieli FK, Ndenga BA, Githure JI, et al. Topography and malaria transmission heterogeneity in western Kenya highlands: prospects for focal vector control. Malar J 2006;5:107.
[20] Abay SM, Tilahun M, Fikrie N, Habtewold A. *Plasmodium falciparum* and *Schistosoma mansoni* co-infection and the side benefit of artemether-lumefantrine in malaria patients. J Infect Dev Ctries 2013;7:468—74.
[21] Rutagwera DG, Tylleskär T. Co-infection with malaria, hookworm and schistosomiasis among school children in Zambezi: a school-based rapid survey. Med J Zambia 2012;39(4):18—23.
[22] Mazigo HD, Waihenya R, Lwambo NJS, Mnyone LL, Mahande AM, Seni J, et al. Co-infections with *Plasmodium falciparum*, *Schistosoma mansoni* and intestinal helminths among schoolchildren in endemic areas of northwestern Tanzania. Parasit Vectors 2010;3:44.
[23] Weber MW, Zimmer Mann U, Van Hens Brock MB, Frenkil J, Palmer A, Ehrich JHH, et al. Renal involvement in Gambian children with cerebral or mild malaria. Trop Med Int Health 1999;4:350—94.

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[24] Nand N, Aggarwal H, Sharma M. Systemic manifestations of malaria. J Indian Acad Clin Med 2001;2:189—94.
[25] Ugwuja EI, Ugwu NC. Abnormal findings on dipstick urinalysis of out-patients with malaria in Abakaliki, Nigeria. J Vector Borne Dis 2011;48:205—9.
[26] Kashif W, Siddiqi N, Dincer HE, Hirsch S. Proteinuria: how to evaluate an important finding. Cleve Clin J Med 2003;70:535—47.
[27] Van Velthuysen MLF, Florquin S. Glomerulopathy associated with parasitic infections. Clin Microbiol Rev 2000:55—66.

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