Helminth infections and micronutrients in children

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CHAPTER 5

Soil-transmitted helminth infections and intestinal and systemic inflammation in schoolchildren

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Abstract

The objective of this study was to assess whether soil-transmitted helminth infections are associated with systemic and local intestinal inflammation in school-age children. In two studies in schoolchildren in Cuba (N=1389) and in Cambodia (N=2471), soil-transmitted helminth infections and calprotectin concentrations were measured in stool samples and acute phase proteins CRP and AGP were measured in blood. Associations between helminth infections and elevated concentrations of CRP, AGP and calprotectin were estimated using multiple logistic regression. The prevalence of elevated CRP concentration (≥5 mg/L) was 5.4% in both populations. Elevated AGP (≥1g/L) was found in 39.5% of the Cambodian children and 6.5% of the Cuban children. Fecal calprotectin was elevated (≥ 50 mg/kg) in 9.4% of the Cambodian children and 1.7% of the Cuban children. Soil-transmitted helminth infections in Cuba were mainly due to *Ascaris lumbricoides* and *Trichuris trichiura*, with prevalences of 5.2% and 3.2%, respectively. In Cambodia, hookworm was the most prevalent species (16.6%). We found no significant associations between elevated concentrations of either acute phase proteins or fecal calprotectin and soil-transmitted helminth infections. We did observe a trend towards an inverse association between elevated CRP and STH infections in both studies. Chronic soil-transmitted helminth infections are not associated with either local intestinal or systemic inflammation. The trend towards less elevated CRP concentration in STH infections may indicate a reduced risk of metabolic inflammatory diseases, which merits further investigation.

Introduction

Soil-transmitted helminth (STH) infections with *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm are related to malnutrition in children[1,2]. Associations of STH infections with both anthropometric indices and micronutrient status have been reported[3-5]. However, much remains unknown about the mechanisms at play. A possible mechanism through which STH infections may impair nutritional status is inflammation. Systemic inflammation stimulates metabolism, thereby increasing nutritional demands, while simultaneously decreasing appetite[2,6,7]. Moreover, systemic inflammation is known to result in intracellular sequestering of micronutrients such as iron, eventually leading to functional iron deficiency[8]. Lastly, local inflammation of the gut mucosa caused by infection can impair the absorption of nutrients[9].

Innate systemic immune responses to infection are combined in the acute phase response (APR). Inflammatory cytokines released at the site of infection induce production of several acute phase proteins by the liver. Acute phase proteins enhance pathogen killing mechanisms such as complement activation. The systemic effects of the APR also result in reduced levels of micronutrients such as vitamin A, zinc and iron in the circulation[7]. The hereby reduced availability of micronutrients to pathogens during an APR is thought to play a role in host defense[10,11]. Despite its name, the APR is not restricted to the acute phase of infection but can linger on in chronic disease or (metabolic) inflammation. In nutritional epidemiology, accounting for the APR is recommended by the World Health Organization when interpreting micronutrient data[12]. The acute phase proteins C-reactive protein (CRP) and alpha-1 acid glycoprotein (AGP) are commonly used markers for inflammation and infection. CRP is quickly raised during acute infections and decreases soon after elimination of the trigger, while AGP remains elevated during recovery[12].

Local inflammation of the gut mucosa may be measured by fecal calprotectin, a relatively new biomarker for intestinal inflammation. Calprotectin is a Toll-like receptor 4 ligand, mainly present in neutrophils (and to a lesser extent in monocytes and macrophages)[13]. Given evidence that stool calprotectin levels are not affected by systemic infections, calprotectin is used as a marker specific for intestinal inflammation[13]. Calprotectin is currently mainly used as a clinical disease marker for inflammatory bowel diseases, but has also been proposed as a possible diagnostic tool for environmental enteropathy in epidemiological studies[9,14,15].

Helminths (STH and schistosomes) are known for their immunomodulatory properties. An extensive body of research has shown that several helminth species actively modulate host immunity by eliciting regulatory and type 2 responses[16,17]. Nevertheless,

research on acute phase responses to helminth infections is lacking. Furthermore, although mucosal damage is often proposed as a consequence of STH infections, any relationship between STH infections and intestinal inflammation has not yet been established[1].

The current study investigates blood concentrations of CRP and AGP and fecal concentrations of calprotectin, and their association with STH infections in Cuban and Cambodian schoolchildren. By combining data from two large studies in populations differing in both STH species prevalence and overall nutritional status, we explore the presence and extent of local intestinal and systemic inflammation in STH-infected and uninfected schoolchildren.

Methods

We used data from two large epidemiological studies on STH infections in Cuba (2009) and Cambodia (2012). The Cuban study took place in the rural municipality of San Juan y Martínez, in the Pinar del Río province. A total of 1389 children was recruited from 13 randomly selected primary schools. Parents or caretakers of the children gave written informed consent. This study was approved by the ethical committees of the Pedro Kourí Institute of Tropical Medicine and the National Institute for Hygiene, Epidemiology and Microbiology in Havana, Cuba and the Institute of Tropical Medicine in Antwerp, Belgium.

The Cambodian study was a randomized controlled trial on the effects of multiple-micronutrient-fortified rice on child nutrition and morbidity from which we used baseline data. The study took place in Kampong Speu province, with baseline data collection in November 2012. From 20 randomly selected primary schools, 2471 children were included. Written informed consent was obtained from parents or caretakers of the children. This study was approved by the Cambodian Ministry of Health, Education and Planning and the Ethical Review board of PATH, USA.

In both studies, STH infection was determined in single fresh stool samples by duplicate 25mg Kato-Katz faecal thick-smear examination[18]. In Cuba, STH infected children were given one single dose of 500 mg mebendazole was given, which is the treatment of choice in Cuba[19]. STH infected children in Cambodia received one single dose of 400 mg albendazole[20]. Height and weight were measured by trained investigators. In Cambodia, weight was measured using a calibrated Body Composition Monitor Scale from Tanita BC-543, Japan to the nearest 0.1 kg. Height was measured by a portable measuring tape (USA) to the nearest 0.1 cm. In Cuba, weight was measured within 0.1 kg with a calibrated electronic flat scale (Seca 888) and height was measured to the nearest 0.1

cm using a portable stadiometer (Seca 225). Height-for-age and weight-for-age z scores were calculated using the WHO 2007 reference curves [21]. In Cuba, 10 ml of venous blood was obtained from participants by venipuncture. Serum high-sensitivity CRP and AGP were measured using an ImmunoTurbidimetric Assay (CPM, Italy). In Cambodia, 5 ml of venous blood was obtained from participants by venipuncture. In 100 ul plasma aliquots, CRP and AGP were measured by sandwich ELISA techniques (VitMin Laboratories, Germany)[22]. In both studies, aliquots of fecal samples were stored at -20˚C and sent to the Institute of Tropical Medicine Antwerp. Calprotectin ELISA kits were used according to the manufacturer's instructions (Calpro, Norway). Calprotectin analyses were done in random subsamples from both study populations. In the Cuban study, AGP was measured in a subsample of 480 children. Elevated concentrations for each inflammatory marker were defined as CRP ≥ 5 mg/L, AGP ≥ 1 g/L[12], and calprotectin ≥ 50 mg/kg[23,24], respectively.

Statistical analysis of the associations between STH and inflammatory markers was done by multiple logistic regression analysis in SPSS version 21 (IBM, New York, USA). In this analysis, the odds of having elevated CRP, AGP or calprotectin were compared between STH infected and uninfected children, adjusted for age in years (as continuous covariate) and sex. Statistical significance was defined as a p value below 0.05.

Results

Prevalence of any STH infection was below 20% in both populations (Table 5.1). *Trichuris trichiura* and *Ascaris lumbricoides* were the most prevalent STH species found in Cuba (5.2% and 3.2%, respectively), while hookworm was more prevalent in Cambodia (16.5%). The Cuban children had above-average height and weight for age, while the Cambodian children were on average much smaller and lighter. In both studies, 5.4% of the children had elevated CRP levels. The percentage of children with elevated AGP concentration was much higher in Cambodia (39.5%) than in Cuba (6.5%). Elevated fecal calprotectin levels were found in 3.7% of the Cuban children and 9.4% of the Cambodian children.

Table 5.2 shows the prevalences and results of the multiple logistic regression analysis of elevated CRP, AGP, and calprotectin within the STH infected and uninfected children. None of the differences in inflammatory markers between STH infected and uninfected children were statistically significant. In both studies, the prevalence of elevated CRP was lower in STH infected children than in those uninfected, almost reaching statistical significance in the Cambodian study population.

Table 5.1. Characteristics of the two study populations.

	Cuba	%	Cambodia	%
N	1389		2471	
Sex (male)	742	53.4	1235	50.0
Age[1]	8.14 ± 2.07		9.68 ± 2.27	
Height-for-age z score[1]	0.06 ± 1.01		-1.81 ± 1.05	
Weight-for-age z score[1,2]	0.15 ± 1.21		-1.88 ± 0.92	
STH uninfected[3]	1261/1379	91.4	1493/1795	83.2
Any STH infection[3]	118/1379	8.6	296/1795	16.8
Ascaris lumbricoides[3]	72/1379	5.2	5/1795	0.3
Light (<5.000 epg)	55	4.1	5	0.3
Moderate (5.000-50.000 epg)	15	1.1	0	0.0
Heavy (>50.000 epg)	0	0.0	0	0.0
Trichuris trichiura[3]	44/1379	3.2	5/1795	0.3
Light (<1.000 epg)	38	2.8	6	0.3
Moderate (1.000-10.000 epg)	2	0.1	0	0.0
Heavy (>10.000 epg)	2	0.1	0	0.0
Hookworm [3]	16/1379	1.2	293/1795	16.3
Light (<2.000 epg)	13	1.0	283	15.8
Moderate (2.000-4.000 epg)	0	0.0	9	0.5
Heavy (>4.000 epg)	2	0.1	1	0.1
CRP ≥ 5 mg/L[3]	73/1356	5.4	130/2396	5.4
AGP ≥ 1 g/L[3]	31/480	6.5	948/2397	39.5
Calprotectin ≥ 50 mg/kg[3]	24/634	3.7	35/371	9.4

[1] mean± sd

[2] for children under 10 years

[3] n/N

Table 5.2. Elevated CRP, AGP and calprotectin levels in STH infected and uninfected children.

		CRP ≥ 5 mg/L		AGP ≥ 1 g/L		Calprotectin ≥ 50 mg/kg	
		n/N (%)	aOR[1] (95%CI)	n/N (%)	aOR[1] (95%CI)	n/N (%)	aOR[1] (95%CI)
Cuba	**STH uninfected**	70/1229 (5.7%)	1 (ref)	27/447 (6.0%)	1 (ref)	22/587 (3.7%)	1 (ref)
	STH infected	3/117 (2.6%)	0.44 (0.14- 1.42)	3/31 (9.7%)	1.54 (0.44- 5.45)	2/42 (4.5%)	1.23 (0.28- 5.46)
Cambodia	**STH uninfected**	84/1426 (5.9%)	1 (ref)	539/1426 (37.8%)	1 (ref)	23/263 (8.7%)	1 (ref)
	STH infected	9/293 (3.1%)	0.52 (0.26- 1.05)	112/293 (38.1%)	1.01 (0.77-1.30)	10/99 (10.1%)	1.21 (0.55- 2.67)

[1] from multiple logistic regression, adjusted for age and sex

Discussion

In this study, we assessed whether STH infections are associated with systemic and local intestinal inflammation in schoolchildren. A series of inflammatory markers was determined across two study populations with different STH species prevalences and overall nutritional status, which provided valuable insights. We found no significant associations of soil-transmitted helminth infections with acute phase proteins or with fecal calprotectin in Cuban and Cambodian schoolchildren. We did observe a trend towards lower CRP concentrations in STH infected children compared to their uninfected peers in both studies.

A recent study in Kenyan preschool children reported a similar trend towards less inflammation (elevated CRP and/or AGP) in STH infection[3]. This negative trend of CRP in STH infected children is of interest, since this inflammatory marker is strongly associated with metabolic syndrome. Increased CRP concentrations are highly predictive of cardiovascular events in adults[25]. In children, CRP is also strongly associated with adiposity and other cardiovascular risk markers[26,27]. The possibility of a negative association between helminth infections and metabolic syndrome is currently an emerging area of research[28]. By suppressing pro-inflammatory immune responses, helminth infections may dampen pathways leading to metabolic syndrome[29]. Further epidemiologic and mechanistic studies are needed to evaluate whether STH infections reduce the risk of metabolic syndrome in humans, similar to what has been found for allergic inflammation[30].

The absence of elevated CRP levels in both study populations is in concordance with the often chronic nature of STH infections. Still, raised AGP would be expected in persistent infections[31,32]. Malnutrition, which has also been associated with STH infections, could be a cause of reduced acute phase responses[33]. However, the Cuban study population was well-nourished but had low levels of AGP, while in the Cambodian study, height and weight-for-age were much lower and elevated AGP was common (Table 5.1). Therefore, the lack of increased AGP in STH infections cannot be attributed to malnutrition in the present study.

A lack of elevated acute phase proteins has also been reported in STH infected children in Indonesia[34] and Zanzibar[35]. Reduced acute phase proteins were recently described in active tuberculosis patients co-infected with STH *Strongyloides stercoralis*[36]. In individuals without tuberculosis infection, this effect was less pronounced. In *Schistosoma japonicum* infections, CRP levels were found to be significantly raised[37]. In *S. haematobium* infected anemic Malian children, infection intensity was positively, but not significantly, correlated with CRP and AGP[38]. *Schistosoma* species differ from STH in

that adult stages reside in the blood stream. For this reason, schistosomes might be more likely to induce systemic immune responses than the gut-dwelling STH.

Our findings confirm those of a previous study in Uganda which found no significant association between fecal calprotectin and intestinal parasitic or bacterial infections [24]. Possibly, local intestinal inflammation in STH infections is very low-grade, or characterized by cell types that do not produce calprotectin. Hookworm can specifically inhibit neutrophils, the main calprotectin-producing cell type[39]. Generally, helminth infections are characterized by eosinophilia, and fecal calprotectin is not elevated in patients with eosinophilic colitis[16,40].

Our study has several limitations. Most STH infections in both study populations were of light intensity, therefore associations of moderate or high infection intensities with inflammatory markers may have been missed. Despite the considerable size of our study populations, the small number of children with elevated markers of inflammation is a limitation to our analysis. We also explored whether other estimates such as mean or median concentrations of the studied markers differed significantly between infected and uninfected children, which was not the case. Lastly, as this is a cross-sectional study, causality cannot be inferred. We do not know whether treatment of STH infections would result in changes in inflammatory markers, or whether newly acquired infections may be associated with a temporary increase in inflammation.

In conclusion, our findings show that chronic STH infections are not associated with markers of systemic inflammation in children. In addition, STH infection was not associated with local intestinal inflammation as measured by fecal calprotectin. Evasion of systemic and local inflammation could contribute to helminth lifespan and fecundity, as well as enhance their access to essential nutrients such as iron[10]. The absence of strong inflammatory responses to helminths in man, and/or the evasion of systemic or local inflammation by helminths might reflect the many centuries of co-evolution of helminths with their human host. The observed trend in STH infections towards lower CRP concentrations and hence possibly a reduced risk of metabolic inflammatory diseases merits further investigation. This is increasingly relevant in the context of countries endemic for STH infections and transitioning towards a chronic disease landscape.

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