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Correlation between eosinophil count and soil-transmitted helminth infection in children

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ABSTRACT

Objective: To assess the prevalence of soil-transmitted helminth (STH) infection and its correlation with eosinophil counts among elementary school children within the Medan Belawan District, North Sumatera Province, Indonesia.
Methods: Ninety-nine subjects participated in this study (ascariasis, $n = 40$; trichuriasis, $n = 9$; mixed infection, $n = 50$). Stool samples were examined by using the Kato-Katz method, and blood samples were examined by using peripheral blood smears.
Results: The prevalence of STH infection in children from two elementary schools was 65.4%; the baseline prevalences of ascariasis, trichuriasis, and mixed infection were 37.1%, 21.9%, and 40.8%, respectively. The respective mean eosinophil counts among children with ascariasis, trichuriasis, and mixed infection were 7.18%, 8.11%, and 8.64% ($P = 0.32$), and the respective prevalences of eosinophilia were 47.5%, 77.8%, and 70% ($P = 0.05$). The eosinophil counts correlated significantly with the number of eggs per gram of feces; ascariasis correlated strongly ($P < 0.001$; $r = 0.62$), and trichuriasis showed correlated weakly ($P = 0.04$; $r = 0.21$) with this parameter. The mean eosinophil count correlated significantly with STH infection intensity ($P < 0.001$).
Conclusions: Eosinophil counts correlated strongly with ascariasis while weakly with trichuriasis. We determined that eosinophil count may be used as a marker of STH infection.

1. Introduction

Approximately 2000 million people with soil-transmitted helminth (STH) infection worldwide experienced severe morbidity, as this infection is associated with 9000–135000 deaths per year[1]. STHs are a group of parasitic nematode worms that cause infection in humans following either the ingestion of eggs or contact with larvae, which develop rapidly in the warm climate and moist soil that are frequently observed in both tropical and subtropical countries worldwide. These worms represent a serious global health problem, particularly in developing countries[2,3]. In Indonesia, STH infection remains a major health problem, particularly in rural areas; however it is still considered as neglected tropical diseases[4]. The three most common types of STH infection are caused by *Ascaris lumbricoides* (*A. lumbricoides*), *Trichuris trichiura* (*T. trichiura*), and hookworm[5].

STH infection is often associated with a significant incidence of eosinophilia worldwide[6]. Increasing levels of eosinophilia may be used as a marker of STH infection[7]. A cross-sectional study in the Philippines observed that eosinophil counts were higher among people suffering from STH infection and were indicative of larger eggs per gram feces (epg) values[8]. The purpose of this study was to assess the prevalence of STH infection and to determine whether a correlation exists between eosinophil counts and STH infection among children in North Sumatera province, Indonesia.

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2. Methods and materials

2.1. Study population

The study included children from two elementary schools (SDN 060969 and SDN 064003) in the Medan Belawan District, North Sumatera Province, Indonesia. The target populations were school-aged children who suffered from STH infection. Children between 6 and 12 years of age were included. Children who suffered from allergies, asthma, atopic dermatitis, rheumatology, malignancy, immunodeficiency or either gastrointestinal, or parasitic infections other than STH were excluded. Additionally, those children who had received antihelminthic drugs within three months of the beginning of the study, or who were taking medications that may have affected their eosinophil counts (*e.g.,* glucocorticoids, myelosuppressive drugs, interferon alpha, antihistamines, cromolyn, cyclosporine, leukotriene inhibitors and antagonists, and phosphodiesterase inhibitors) were also excluded. Written informed consent was provided by each child's parents. The study was approved by the Research Ethics Committee of the Medical School of the University of Sumatera Utara.

2.2. Study design

This observational study had a cross-sectional design that included school-aged children and took place between February and March of 2014. The subjects' characteristics and clinical data were obtained via questionnaires, whereas their histories of previous illnesses and medication usage were obtained by interviewing each child's parents. Numbered stool containers were distributed to the children who met the study's inclusion criteria. Children were asked to fill the stool container the following day. The stool samples were collected in the morning. The Kato-Katz method was used to screen the samples for STH infection[9]. The children who suffered from STH infection underwent subsequent blood analyses and anthropometric measurements. Two blood specimens were collected from the subjects at noon (10 am until 12 pm). The subjects' eosinophil counts were examined using peripheral blood smears, and eosinophilia was examined using an improved Neubauer cell counting chamber to determine the subjects' white blood cell counts. All stool samples and blood specimens were analyzed by an experienced analyst from the pediatric clinical laboratory of Haji Adam Malik Hospital. The eosinophil count was defined as the number of eosinophils within the peripheral blood as determined via the peripheral blood smear analysis. The stool samples were sub-classified based on the number of epg. A mild infection of *A. lumbricoides* was diagnosed for an epg level of 1 to 4999; a moderate infection was diagnosed for an epg level of 5000 to 49999; and a severe infection was diagnosed for an epg level of > 50000[10]. A mild infection of *T. trichiura* was diagnosed for an epg level of 1 to 999 epg; a moderate infection was diagnosed for an epg level of 1000 to 9999; and a severe infection was diagnosed for an epg level of > 10000[10]. Eosinophilia was defined as an increase in the eosinophil count of > 450 eosinophils/μL as determined via peripheral blood sampling[11]. All subjects received a single dose of albendazole, 400 mg. Anthropometric measurement was conducted to assess nutritional status. Weight was measured using a Camry weight meter (sensitivity up to 0.5 kg) and height was measured using WESMEDIC microtoise (sensitivity up to 0.5 cm). Nutritional status was determined using the The National Center for Health Statistics (NCHS)-World Health Organization (WHO) anthropometric reference. It was classified based on the indexes of weight and height ratio using Centers for Disease Control and Prevention 2000 chart. Weight for height were considered as obese if the ratio was > 120%; overweight if the ratio was 110%–120%; normal if the ratio was 90%–110%; mild malnutrition if the ratio was 80%–90%; moderate malnutrition if the ratio was 70%–80%; severe malnutrition if the ratio was < 70%[12].

2.3. Statistical analysis

The data are presented as means, percentages, and standard deviations. The statistical analysis was performed using a Pearson correlation to determine the correlation between the eosinophil counts and STH infection. The regression equation correlation between eosinophil counts and epg was evaluated using a linear regression equation. This study used a 95% confidence interval; a *P* value < 0.05 denoted significance. All statistical analyses were performed using SPSS, version 19.0.

3. Results

Six-hundred-fifty-eight school-aged children were screened in this study. No stool samples were submitted by 74 children therefore, 584 stool samples were investigated using the Kato-Katz method. Based on these samples, 382 children suffered from an STH infection (65.4%), including 142 children who were infected by *A. lumbricoides* (37.1%), 84 children who were infected by *T. trichiura* (21.9%), and 156 children who were infected by both *A. lumbricoides* and *T. trichiura* (40.8%). Unfortunately, 283 children did not provide blood samples for analysis. Therefore, only 99 children completed the entire battery of tests utilized in this study.

Based on the characteristics of the subjects studied, no significant difference existed among the groups (Table 1). The ages of the subjects ranged between 7 and 13 years. We did not observe any infections of severe intensity during this study.

Table 1
Characteristics of subjects.

Characteristics		Ascariasis (*n* = 40)	Trichuriasis (*n* = 9)	Mixed infection (*n* = 50)
Gender [*n* (%)]	Male	22 (55)	3 (33.3)	21 (42.0)
	Female	18 (45)	6 (66.7)	29 (58.0)
Age (years), mean (SD)		9.1 (1.5)	8.4 (1.6)	9.3 (1.5)
BW (kg), mean (SD)		23.8 (8.1)	20.6 (4.3)	23.3 (5.9)
BH (cm), mean (SD)		124.5 (11.5)	119.6 (11.0)	123.7 (11.3)
Nutritional status [*n* (%)]	Normal	19 (47.5)	6 (66.7)	30 (60.0)
	Mild Malnutrition	12 (30)	2 (22.2)	13 (26.0)
	Moderate Malnutrition	4 (10.0)	1 (11.1)	5 (10.0)
	Overweight	3 (7.5)	0 (0.0)	1 (2.0)
	Obesity	2 (5.0)	0 (0.0)	1 (2.0)
Intensity of infection [*n* (%)]	Mild	37 (92.5)	8 (88.9)	38 (76.0)
	Moderate	3 (7.5)	1 (11.1)	12 (24.0)
	Severe	0 (0.0)	0 (0.0)	0 (0.0)

BW: Body weight; BH: Body height.

The mean eosinophil count was not significantly different between the patients who suffered single infections and those who suffered mixed infections (*P* = 0.32), as demonstrated in Table 2. The prevalences of eosinophilia among the children with ascariasis, trichuriasis, and mixed infection were 47.5%, 77.8%, and 70.0%, respectively (*P* = 0.05) as demonstrated in Table 2. This finding suggests that trichuriasis had the highest prevalence of eosinophilia compared with both ascariasis and mixed infection.

Table 2
Eosinophil count and absolute eosinophil count in STH infection.

STH infection	Eosinophil count (%)				P^a	Absolute eosinophil count (eosinofil/μL blood)		P^b
	Mean	SD	95% *CI*			≤ 450	> 450	
			Lowest	Highest		*n* (%)	*n* (%)	
Ascariasis	7.18	2.66	6.32	8.03		21 (52.5)	19 (47.5)	
Trichuriasis	8.11	2.42	6.25	9.97	0.32	2 (22.2)	7 (77.8)	0.05
Mixed infection	8.64	3.30	7.70	9.58		15 (30.0)	35 (70.0)	

CI: Confidence interval; a: Anova is used to analyze the difference of eosinophil count and soil-transmitted helminth infection; b: *Chi*-squared test is used to analyze the difference between absolute eosinophil count and soil-transmitted helminth infection.

The mean eosinophil count in mild and moderate intensity of STH infection was 7.11% and 12.63% (*P* < 0.001). Higher mean eosinophil counts were noted in conjunction with STH infections of increased intensity. We noted a strong correlation for ascariasis (*P* < 0.001; *r* = 0.62) and a weak correlation for trichuriasis (*P* = 0.04; *r* = 0.21).

The eosinophil percentage correlated positively with egg in the setting of STH infection, indicating that epg increased as the eosinophil count increased. The results of the regression analysis predicted the incidence of isolated *A. lumbricoides* eggs (slope = 615.141; *P* = 0.01), isolated *T. trichiura* eggs (slope = 95.090; *P* = 0.12), *A. lumbricoides* eggs in the setting of mixed infection (slope = 2230.692; *P* < 0.001), and *T.*

trichiura eggs in the setting of a mixed infection (slope = 2593; P = 0.77). An increase in the eosinophil count of 1% was predictive of increase in isolated *A. lumbricoides* eggs, isolated *T. trichiura* eggs, *A. lumbricoides* eggs in the setting of a mixed infection and, *T. trichiura* eggs in the setting of a mixed infection, with increases of 615.141 epg, 95.090 epg, 2230.692 epg, and 2593 epg, respectively (Figures 1–3). The R^2 values of isolated *A. lumbricoides* eggs, isolated *T. trichiura* eggs, *A. lumbricoides* eggs in the setting of a mixed infection, and *T. trichiura* eggs in the setting of a mixed infection indicated that 17%, 21%, 51.6%, and 2% of the total variations in epg may be explained by variations in eosinophil percentages.

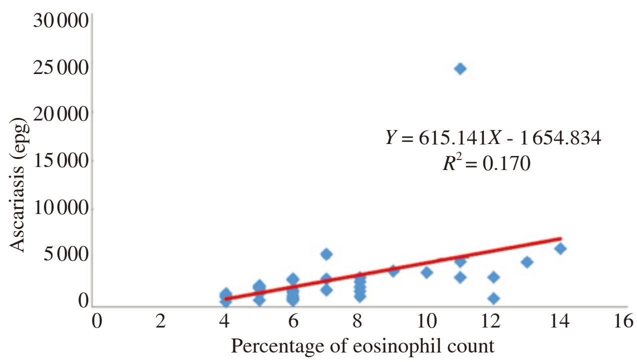

Figure 1. The correlation between the percentage of eosinophils and epg for ascariasis.
The red line depicts the correlation.

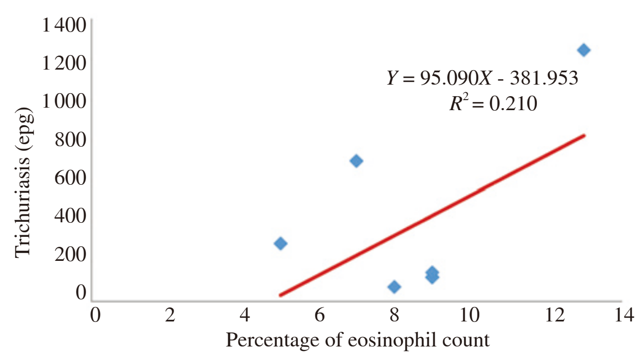

Figure 2. The correlation between the percentage of eosinophils and epg for trichuriasis.
The red line depicts the correlation.

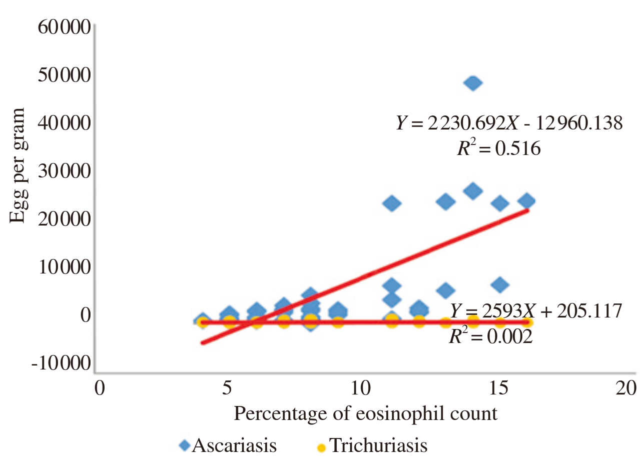

Figure 3. The correlation between the percentage of eosinophils and epg for mixed infection.
The red line depicts the correlation.

4. Discussion

STH infection remains a major health problem in many poor and developing countries[13,14]. Consistent with the findings of previous studies, our study confirmed that STH infection remains a problem in children from two elementary schools at Medan Belawan District, North Sumatera province, Indonesia with a prevalence of 65.4%.

A cross-sectional study in Honduras reported that the mean levels of eosinophil counts observed in the setting of ascariasis, trichuriasis, and mixed infection were 6%, 5.2%, and 7.2%, respectively[15]. Another study in the Philippines observed higher mean levels of eosinophil counts, 10%, 13%, and 15.8%, respectively (P = 0.353)[8]. The mean levels of eosinophil counts observed in our study were 7.18%, 8.11%, and 8.64% (P = 0.32), respectively. There were no significant differences between individual infections and mixed infections.

Although eosinophilia has many causes, including infections and malignancies, it is often caused by tissue-helminth infections[16]. During STH infection, the response by the $CD4^+$ T-helper type 2 cells stimulates excessive eosinophilia via cytokine activity[17]. Eosinophilia is characteristics of the host immune response to helminthiasis, particularly in the invasive tissue stage of the infection[18]. It may be harmful because of its proinflammatory effects, but it may also be helpful because of its antiparasitic effects[19,20].

A retrospective investigation in Germany involving 14298 travellers who returned from developing countries described 29 patients who were infected with ascariasis and 27 patients who were infected with trichuriasis. Schulte *et al.* determined that the prevalence of eosinophilia was 24.1% for ascariasis and 14.8% for trichuriasis[7]. One case report from Kyushu, Japan described marked eosinophilia (30%–70%) in the setting of ascariasis[21]. However, we observed that the highest prevalence of eosinophilia occurred in the setting of trichuriasis (77.8%) compared with mixed infection (70.0%) and ascariasis (47.5%) (P = 0.05). Further studies should be considered because of a small number of trichuriasis samples.

A previous study in the Phillipines described a moderate correlation (r = 0.328; P = 0.004) between percentage of eosinophil count and STH infection[8]. In our study, the correlation between eosinophil count and epg was indicative of a high correlation in the setting of ascariasis (r = 0.62; P < 0.001) and a low correlation in the setting of trichuriasis (r = 0.21; P = 0.04). The level of eosinophil correlated significantly with the intensity of the infection for both ascariasis and trichuriasis (P < 0.001). This finding indicated that the eosinophil count may be used as a marker of both STH infection and STH infection severity.

The accuracy of the Kato-Katz technique in identifying individuals with an STH infection was limited by variations in worm egg excretion over several days; it was difficult to distinguish STH infection from infections caused by other types of parasites, because doing so depends on the experience of the laboratory technicians[9]. Improvement may be facilitated by obtaining multiple stool samples on consecutive days, and by utilizing different diagnostic techniques[22].

Several limitations to our study have been identified. First, information recall bias from both the questionnaires and the interviews may have occurred, as some of the information obtained was based on memory. Second, the difficulties in obtaining approval for venous blood sampling may have affected our result. Third, we used single stool samples and only the Kato-Katz technique.

A randomized controlled trial study of 155 children in South Africa demonstrated a significant reduction in mean eosinophil counts following the administration of six doses of an antihelminthic over two years[23]. Additional studies should be performed on a larger scale with a prospective cohort to determine patients' eosinophil counts after administering antihelminthic therapy, which may have positive implications in re-balancing patients' immune profiles and serve as an indicator of disease improvement.

In conclusion, eosinophil counts correlated strongly with ascariasis and weakly with trichuriasis. They may be used as markers of STH infection in the North Sumatera Province, Indonesia.

Conflict of interest statement

We declare that we have no conflict of interest.

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