Correlation between Soil Transmitted Helminth Infection and Eosinophil Levels among Primary School Children in Medan

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Abstract

BACKGROUND: Soil Transmitted Helminth infection is one of most prevalent health problems worldwide, especially in environments with poor sanitation. Based on World Health Organisation (WHO) data, more than 2 billion people, or 24% of the world's population, are infected with intestinal parasite. The highest prevalence is located in areas of poor sanitation and unsafe water supplies. In Indonesia, the prevalence of parasite infections is 15% of the entire population.

AIM: The purpose of this study was to determine the relationship between Soil Transmitted Helminth infection on levels of eosinophils among primary school children. In addition, this study also aimed to determine the prevalence of different types of worm infections and the levels of eosinophils in children infected with worms.

MATERIAL AND METHODS: This study was analytic observational using a cross-sectional method. The sampling technique was consecutive and in total 132 samples was obtained. The study involved primary school children in Amplas Medan and Hamparan Perak, Deli Serdang through May to October 2016. Univariate analysis was performed to determine STH infection prevalence and bivariate analysis was used to find the correlation between STH infection and eosinophil levels through a Chi square ($\chi^2$) test.

RESULTS: The results showed that the prevalence of Soil Transmitted Helminth was 7.6%. The most common types of STH infection were 3.8% with Trichinosis trichiura and 3% with Ascaris lumbricoides. A significant correlation was found between Parasite infection and eosinophil levels (Contingency Coefficient (C) = 0.2, $\chi^2 = 5.3$, p = 0.021) and the risk of STH infection that caused eosinophilia or increased eosinophil levels in the children with a Prevalence Ratio (PR) of 1.56 (Confidence Interval (CI) 95%: 1.10-2.22).

CONCLUSION: It is recommended that schools at similar risk improve and maintain hygiene and healthy behaviour in the school environment and that parents and teachers pay greater attention to the cleanliness of their children.

Introduction

Soil-transmitted helminth (STH) infection is the most prevalent health problem in the world, especially in areas with bad hygiene. STH is the group of parasitic worms that cause infection in humans, especially children, and which spread through soil contaminated with worm eggs. The geographical location of Indonesia with its tropical climate is suitable for the development of STH. This includes a group of STHs, roundworm (Ascaris lumbricoides), whipworm (Trichuris trichiura) and hookworm (Ancylostoma duodenale and Necator americanus) [1].

Around the world, more than 2 billion people, or 24% of the world's population, are infected with STH. The highest prevalence is located in areas of poor sanitation and unsafe water supplies. Infection is distributed in tropical and subtropical regions with the largest numbers being in sub-Saharan Africa, America, China and East Asia [2].
The prevalence of infection of STH ascariasis is about 807.1 million, trichuriasis around 604-795 million, and hookworm approximately 576-740 [3]. The global prevalence of Strongyloides stercoralis is not known, but experts estimate that there are 30-100 million people infected throughout the world [4]. In Indonesia, the prevalence of STH infection is 15% of the entire population [5], while its prevalence in Belawan and Amblas Medan, North Sumatra is 65.4% and 40.3%, respectively [6, 7].

STH infections are common in children of primary school age because their habits and behaviours can be unhygienic, causing them to be more easily infected by worm eggs in the soil. More than 270 million preschool children and more than 600 million school-age children live in areas where the parasite is intensively transmitted, and they are in need of treatment and prevention [2].

STH infection is often associated with a significant incidence of eosinophilia worldwide [8]. An eosinophil is a type of white blood cell. Increasing levels of eosinophil may be used as a marker of STH infection [9]. A normal eosinophil count is 0-6% of the total leukocyte count (TLC) [10]. Eosinophil increases will cause eosinophilia, which is defined as eosinophil levels of more than 600 μL in the blood or more than 6% of the TLC [11].

Eosinophils can be increased by several diseases, such as allergies, asthma, atopic dermatitis, rheumatic diseases, malignancies, immunodeficiency, or either gastrointestinal or parasitic infections, including STH infection [9, 12, 13].

Several studies have shown a correlation between STH infection and eosinophils. There is a relationship between STH infection with significant levels of eosinophils and the prevalence of STH infections, including eosinophilia ascariasis (47.5%), trichuriasis (77.8%), and mixed infections (70%) [6].

Based on the studies above, this research was conducted on the correlation between STH infection and eosinophil levels among children of primary school age. The main goal was to determine the correlation between STH infection and eosinophil levels in students at public primary schools in Medan.

Materials and Methods

This research is a cross-sectional analytical study to determine whether or not there is a correlation between STH infection and the levels of eosinophils in children. This study was conducted between May and October 2016 at Public Primary School 060925 Harjosari 1, Amblas Medan and Public Primary School 101747 Hamparan Perak, Deli Serdang (Figure 1). The reason for this choice of locations was their moist and fertile soil as well as the sheer lack of cleanliness of the school environments and the lack of personal hygiene in the students. Samples were taken from subjects of the population who met all the inclusion criteria and had no exclusion criteria. The consecutive sampling technique was used. The inclusion criteria were primary school students from grades III to VI aged 8-12 years, who were willing and had their parents’ approval to participate. They needed to provide a stool sample and be willing to have blood drawn. They were also children who had not undertaken anthelmintic treatment in the previous six months and had no history of allergy, asthma, atopic dermatitis, rheumatic disease, immunodeficiency, malignancies, infectious diseases, other parasites or a history of haemophilia. Exclusion criteria were stool and blood samples that were damaged or missing.

The study protocol was approved and granted by the Ethics Committee of the Faculty Medicine of University Sumatera Utara. Informed consent was taken before enrolling the children into the study. The procedure was fully explained to the parents and consent received before the procedures are done. Permission was also taken from the primary school authority.

In this study, information about the characteristics and history of the disease and the drug consumption of the respondents was obtained from a form filled out by their parents. Data was collected by examination of stools and peripheral blood smears. Stool examination was done to confirm the diagnosis of worm infections by finding eggs or larvae in the faeces. Stool examination using the Kato-Katz technique comprised qualitative and quantitative methods. The stool samples were collected by pot stool in the morning. Faeces were preserved in 10% formalin for parasites to be transported to the laboratory where the stool was stained and examined under a microscope [14, 15]. The stool samples were subclassified into Ascaris lumbricoides, Trichuris trichiura and hookworm, based on worm egg morphology. Peripheral blood sampling was also carried out to check the levels of eosinophils in the
blood. Blood samples were taken using a venipuncture technique [16]. Eosinophil levels were examined using an improved Neubauer chamber to determine the subjects’ white blood cell counts in peripheral blood smears [6]. The results of the eosinophil count were grouped into normal and eosinophilia. An eosinophil count of 0-6% of total leukocytes was called normal, and an eosinophil count of > 6% total leukocytes was called eosinophilia [10, 11]. Statistical analysis of the data was performed using Predictive Analytics Software (PASW) for Mac version 20. Frequencies were used to describe variable distributions. Chi-square ($\chi^2$) was used in bivariate analyses of prevalence and correlation of STH infection and eosinophilia. The test was considered significant at $P < 0.05$ (CI 95%).

### Results

In total, 132 students aged 8-12 years old and in grades 3, 4, 5 and six from Public Primary School 060925 Harjosari 1, Amplas Medan and Public Primary School 101747 Hamparan Perak, Deli Serdang, and who fulfilled the criteria, participated in this study. Of the 132 students, 54.5% were boys, and 45.5% were girls. Based on age and grade, the largest groups in this study were 12 years old at 32.6% (43/132) and in grade 4 at 37.88% (50/132) (Table 1).

Based on this study, only 7.6% students were found to be positive for STH infection, 3% with *Ascaris lumbricoides*, 3.8% with *Trichuris trichiura*, and 0.8% with hookworm infection. The prevalence of eosinophilia was 24.2% (Table 1).

#### Table 1: General characteristics of the children participating in the study

| Gender | Boys | Girls |
|--------|------|-------|
| Age    | 8    | 9     | 10   | 11   | 12   |
| 8      | 4 / 3.0 | 18 / 3.6 | 34 / 25.8 | 33 / 25.0 | 43 / 32.6 |
| 9      |      |       |      |      |      |
| 10     |      |       |      |      |      |
| 11     |      |       |      |      |      |
| 12     |      |       |      |      |      |
| Class  | 3    | 4     | 5    | 6    |      |
| 3      | 5 / 3.79 | 50 / 37.88 | 32 / 24.24 | 45 / 34.09 |      |
| 4      |      | 1 / 0.8 |      |      |      |
| 5      | 32 / 24.2 |      |      |      |      |
| 6      | 45 / 34.09 |      |      |      |      |
| STH Infection |  |      |      |      |      |
| A. lumbricoides | 4 / 3.0 |      |      |      |      |
| Hookworm | 1 / 0.8 |      |      |      |      |
| T. trichiura | 5 / 3.8 |      |      |      |      |
| Eosinophilia (>6 μg/dL) |      |      |      |      |      |
| Yes    | 32 / 24.2 |      |      |      |      |
| No     | 100 / 75.8 |      |      |      |      |

Table 2 shows the prevalence of eosinophilia was higher among the children aged ≤10 years compared with those aged > 10 years (59.4% vs. 40.6%). The children aged ≤10 years had 1.98 times increased risk of eosinophilia (95% CI = 1.07-3.67) compared to the children aged > 10 years. Based on this, there was a significant difference between the age of the children and eosinophilia, though the correlation was weak ($P = 0.026$, $r = 0.19$). Similarly, the children who tested positive for STH infection had a significantly higher prevalence of eosinophilia when compared with their counterparts with weak correlation ($\chi^2 = 3.91$, $P = 0.048$, $r = 0.17$). The children with STH infection had 2.26 times increased risk of eosinophilia (95% CI = 1.12 – 4.57) compared to the children who tested negative for STH infection. Based on gender, there was no significant difference between eosinophilia and gender (RP = 0.65, 95% CI: 0.35-1.19, p > 0.05, $P = 0.159$).

#### Table 2: Levels of eosinophil among the school children according to age and gender (n = 132)

| Variable | Eosinophil Mean ± SD | Eosinophilia (> 6 μg/dL) n (%) | STH Infection n (%) |
|----------|----------------------|--------------------------------|---------------------|
| Age      |                      |                                |                     |
| 8        | 5.65 ± 2.73          | 9 (69)                         | 2 (15.6)            |
| 9        | 4.75 ± 2.15          | 6 (46)                         | 3 (23)              |
| 10       | 5.84 ± 1.22          | 12 (92)                        | 3 (27)              |
| 11       | 3.96 ± 2.78          | 5 (38)                         | 1 (7)               |
| 12       | 4.55 ± 3.92          | 8 (62)                         | 3 (30)              |
| Gender   |                      |                                |                     |
| Boys     | 5.6 ± 4.92           | 18 (66.7)                      | 4 (40)              |
| Girls    | 3.6 ± 2.55           | 14 (46.1)                      | 6 (60)              |

The levels of eosinophil among the children according to age and gender are presented in Table 2. The mean eosinophil level of the boys was 5.6 ± 4.92, significantly higher when compared with the girls at 3.6 ± 2.55; $t = -2.73$, $P = 0.008$). However, the mean level of eosinophil based on the ages of 8-12 years old had no significance when compared ($F = 0.912$, $P = 0.459$). Overall, 7.6% (10/132) of the children were positive for STH infection. The prevalence of STH infection was higher among the girls compared to the boys (60% vs. 40%; $P = 0.755$). Based on age, the prevalence of STH infection was higher among the students aged ≤10 years compared with > 10 years (60% vs. 40%; $P = 0.322$). The prevalence of eosinophilia was higher among the boys compared to the girls (56.3% vs. 43.8%; $\chi^2 = 1.98$, $P = 0.159$). Similarly, with STH infection, based on age the prevalence of eosinophilia was higher among the students aged ≤10 years compared to >10 years (59.4% vs. 40.6%; $\chi^2 = 4.97$, $P = 0.026$) (Table 2).

#### Table 3: Univariate analysis of factors associated with eosinophil levels among primary school children in Medan (n = 132)

| Variable | Examined | Eosinophilia (> 6 μg/dL) n (%) | RP (95% CI) | r | P Value |
|----------|----------|--------------------------------|-------------|---|---------|
| Age      |          |                                |             |   |         |
| ≤10      | 56       | 19 (37.9)                      | 1.98 (95%: 1.07-3.67) | 0.19 | 0.026* |
| >10      | 76       | 37 (49)                        |              |   |         |
| Gender   |          |                                |             |   |         |
| Boys     | 60       | 14 (23.3)                      | 0.65 (95%: 0.35-1.19) | 0.12 | 0.159 |
| Girls    | 72       | 26 (36.1)                      |              |   |         |
| STH Infection | | | | | |
| Yes      | 10       | 5 (50)                         | 2.26 (95%: 1.12-4.57) | 0.17 | 0.048* |
| No       | 122      | 27 (22.1)                      |              |   |         |

RP: Ratio Prevalence; CI: Confidence Interval; * Significant Association ($P < 0.05$).
Discussion

Eosinophilia is a marker of STH infection, and in this study the correlation between STH infection and eosinophilia was significant. The prevalence of STH infection among the children (7.6%) was lower than the previous study conducted in Belawan, Medan in 2015 (40.3%) \[7\]. This may be due to the change in behaviour after education about personal hygiene and prevention of parasite infections, which improved the children’s habits.

Compared to the previous study conducted in Belawan, Medan, the correlations between STH infection and increased eosinophil levels are significant with an STH infection prevalence of 65.4%. The prevalence of eosinophilia among the children who had STH infection with *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm infections was 47.5%, 77.8% and 70%, respectively. The results showed that the high prevalence of STH infection and increased eosinophil levels have a significant correlation and eosinophilia can be used as a marker for STH infection.\[6\] This study was similar to another finding in Honduras. The prevalence of ascariasis, trichuriasis, and hookworm were 24%, 44.3%, and 12%, respectively \[17\]. Both of these studies showed the strong correlation and significant difference between eosinophil and STH infections.

In this study, it was shown that there were 10 cases of positive STH infection (7.6%). The limitation of this study is that there were very few cases of STH infection. The incidence of parasite infection in the study area had decreased compared with the previous study. The result suggested that the correlation between STH infection and eosinophilia was significant. Education and counselling in the prevention of parasite infection had been given in the interim. This showed that the school children’s behaviour had changed so that it was better than it had been before and was maintaining hygiene.

While eosinophilia could be caused by infection and malignancies, it is frequently caused by STH infection. The eosinophils stimulate host defence against helminths, occurred mainly from histologic images of eosinophils and parasites in tissue specimens and in vitro studies that recognised the anti-parasitic activities of eosinophil granule protein \[18,19\].

Based on the results, the prevalence of STH infection among the school children was low. About 7.6% children tested positive for STH infection, which was caused by *Ascaris lumbricoides* (3.0%), *Trichuris trichiura* (3.8%) and hookworm (0.8%). The correlation between STH infection and eosinophil levels was significant (\( \chi^2 = 3.91, p = 0.048 \)). Children with STH infection had 2.26 times increased the risk of eosinophilia (95%: CI = 1.12 – 4.57) compared to the children who tested negative for STH infection.

It is recommended that schools at similar risk improve and maintain hygiene and healthy behaviour in the school environment and that parents and teachers pay greater attention to the cleanliness of their children.

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Reference

1. Brunette GW, editor. CDC Health Information for International Travel 2016: Helminths-Soil-Transmitted. New York: Oxford University Press, 2016: p. 188.
2. World Health Organization. Soil-Transmitted Helminth Infections. Geneva: World Health Organization, 2016.
3. Center for Disease Control and Prevention. Parasites – Soil-Transmitted Helminths (STHs). Atlanta: CDC, 2013.
4. Center for Disease Control and Prevention. Parasites – Strongyloides. CDC, 2014.
5. World Health Organization. Soil-Transmitted Helminthiases: eliminating soil-transmitted helminthiasis as a public health problem in children: progress report 2001-2010. Geneva: World Health Organization, 2012.
6. Jiero S, Ali M, Pasaribu S, Pasaribu AP. Correlation between eosinophil count and soil-transmitted helminth infection in children. Asian Pac J Trop Dis. 2015; 5(10):813-816.
7. Darlan DM, Kaban FFV. Correlation between Soil Transmitted Helminth Infection and Incidence of Anemia at Public Primary School 060925. Int Jour of PharmTech Research. 2016;9(6):185-190.
8. Nutman T.B. Evaluation and differential diagnosis of marked, persistent eosinophilia. Immuniol Allergy Clin North Am. 2007; 27(3): 529–549. https://doi.org/10.1016/j.iac.2007.07.008 PMid:17868863 PMCid:PMC2099264
9. Schulte C, Krebs B, Jelinek T, Nothdurft HD, von Sonnenburg F, Löscher T. Diagnostic significance of blood eosinophilia in returning travelers. Clin Infect Dis, 2002; 34: 407-11. https://doi.org/10.1086/338026 PMid:11753824
10. Kemenkes RI. Pedoman Interpretasi Data Klinik. Jakarta: Kemenkes RI, 2011.
11. Singh V, Gomez VV, Swamy SG, Vikas B. Approach to a Case of Eosinophilia. JASM. 2009; 53(2): 58-64
12. Sutedjo AY, Buku Saku Mengenal Penyakit Melalui Hasil Pemeriksaan Laboratorium: Pemeriksaan hematologi. Yogyakarta: Amara Books, 2012: p. 20-33.
13. Hoffbrand AV, Moss PAH. Kapita Selekta Hematologi edisi 6. Jakarta: EGC, 2013. PMid:24224721
14. Garcia LS. Collection, Preservation, and Shipment of Fecal Specimens. Chapter 26, In Diagnostic Medical Parasitology 5th Ed.
15. Kemenkes RI. Pedoman Pengendalian Cacing, dalam Surat Keputusan Menteri Kesehatan No: 424/MENKES/2006. Jakarta: Kemenkes RI, 2006.

16. World Health Organization. WHO guidelines on drawing blood: best practice in phlebotomy. Geneva: World Health Organization, 2010.

17. Kaminsky RG, Soto RJ, Canda A, Baum MK. Intestinal parasitic infections and eosinophilia in an human immunodeficiency virus positive population in Honduras. Mem Inst Oswaldo Cruz. 2004; 99(7): 773-8. https://doi.org/10.1590/S0074-02762004000700020 PMid:15654438

18. Rosenberg HF, Dyer KD, Foster PS. Eosinophils: changing perspectives in health and disease. Nat Rev Immunol. 2013; 13: 9-22. https://doi.org/10.1038/nri3341 PMid:23154224 PMcid:PMC4357492

19. Simon D, Wardlaw A, Rothenberg ME. Organ-specific eosinophilic disorders of the skin, lung, and gastrointestinal tract. J Allergy Clin Immunol. 2010; 126(1): 3-13. https://doi.org/10.1016/j.jaci.2010.01.055 PMid:20392477 PMcid:PMC2902687