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Comparison of Hepcidin Levels in Children with and without Soil-Transmitted Helminths Infection

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ABSTRACT

Helminths infection is one of the diseases that still occur insociety. The helminth infection caused by the Soil-Transmitted Helminths (STHs) group, which is *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm in human can cause chronic bleeding resulting in decreasir on storage in the body and increased level of hepcidin. Hepcidin is a liver hormone which regulates iron metabolism and can function as marker of inflammation and iron deficiency. This study aimed to compare the hepcidin levels in STH-infected and non-infected children. A cross-sectional study was conducted between May and October 2018 on 28 STH infected and 140 non-infected subjects. The collected stool samples were analyzed using the Kato-Katz method to determine the presence of STH infection and the degree of infection. Urine samples were processed, and their hepcidin levels were measured using a Sandwich-ELISA method. Measurement was made using a Spectrophotometer. The difference of numeric variables was analyzed using Wilcoxon test. The prevalence of STH infection was 16.66%. The prevalence of *Trichuris trichiura* 10.71%, *Ascaris lumbricoides* 4.76% and hookworm 2.97%. The prevalence of a single infection was 14.88% and mixed infection 1.78%. Based on the intensity of infection, 15.48% of subjects were mild infection, 0.59% moderate infection, and 0.59% severe infection. Hepcidin levels in the infected and uninfected group did not differ significantly (p=0.978). There were no different hepcidin levels in children with and without soil-transmitted helminths infection.

Keywords: Hepcidin, soil-transmitted helminths, infection, inflammation

INTRODUCTION

Helminth infection isone of the diseases that still occurs in society despite gaining fewer attention diseases. Under-attention diseases do not cause a sudden outbreak, but it slowly worsens human health, causing permanent disability, decreasing the intelligence of children, and ultimately can also cause death.[1] Helminth infection caused by the Soil-Transmitted Helminths (STH) group, which life cycles through the soil, is one of the neglected diseases.[2] There are five species helminths included in the STH group, which are still a health problem, namely *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*, and hookworm.[1,3] The prevalence of helminth infection in Indonesia is still relatively high. The results of the helminthiasis survey in primary schools of 27 Indonesian provinces in 2002–2006 showed that in 2006, the prevalence of *Ascaris lumbricoides* was 17.8%, *Trichuris trichiura* 24.2% and *Ancylostoma duodenale* 1.0%.[4] These helminth infections can lead to a decrease in health, nutrition, intelligence, and patient productivity due to loss of carbohydrates, proteins, and blood, which ultimately reduces the quality of human resources. Loss of blood due to helminthiasis is a contributing factor to anemia. Human helminthiasis by *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm can cause chronic bleeding, which results in decreased iron storage of the body and ultimately causes iron deficiency anemia. Larocque *et al*. study found that 47.22% of pregnant females with anemia were infected by hookworm, and 82.25% were infected by *Trichuris trichiura*.[5] Likewise, Nurdiati *et al.* in Purworejo District, Central Java, found that 69.7% of pregnant females with anemia were infected with at least one STH species.[6] This study also found that the helminth species that most often infected were *Trichuris trichiura, Necator americanus* (hookworm), and *Ascaris lumbricoides*.[3] Hookworms suck more blood than *Trichuris trichiura*. A moderate infection will cause blood loss at an average of 8 mL per day and so will cause symptoms of anemia and iron deficiency.[7]

Various ways were developed to be able to evaluate iron status in the body. The conventional

method is done by measuring the levels of Serum Iron (SI), Serum Ferritin (SF) and Saturation Transferrin (ST).[8] A recent study showed a new parameter for assessing iron status, namely hepcidin. Hepcidin is a hormone produced in the liver that plays a role in iron metabolism. Normal level of hepcidin is 27-158 ng/mL.[9] According to Wysocka and Turowski in Ginting, there were erythropoiesis disorder in the bone marrow because iron deficiency resulted in decreased hemoglobin synthesis in erythrocyte precursor cells, the reticulocytes, causing the hemoglobin levels to become low.[10]

The relationship between hepcidin and iron metabolism was first stated by Pigeon *et al*. who found that hepcidin mRNA produced by hepatocytes and lipopolysaccharides as well as excessive iron load both orally and parenterally can stimulate the production of hepcidin as a feedback on the state of excessive iron load.[11] In a study by Zaritsky, who examined the relationship of serum hepcidin with indicators of anemia, iron status, inflammation and kidney function.[12] Hepcidin could be used as a biochemical parameter in iron deficiency anemia and was one of the causes of inadequate response of erythropoetin. According to Cherians *et al.* it was found that urinary hepcidin levels were significantly lower in iron deficient state and iron deficiency anemia, but there was no association between urinary hepcidin levels and helminth infection.[13] Whereas according to Ayoya, urine hepcidin levels were positively correlated with inflammatory markers, CRP and serum ferritin, but not significant for hemoglobin levels in school children infected with *Schistosoma haematobium*.[14] Numerous studies have shown that *S.mansoni* products induced IL-10 production by DCs and had a direct anti-inflammatory effection DCs by controlling TLR ligand-induced DC maturation. *S.mansoni* had also been shown to induce alternatively activated Macrophage, which secrete small amounts of inflammatory mediators and inhibit T-cell proliferation.[15] Inflammation causing an increase in levels hepcidin rapidly and hypoferemia consequently, with interleukin-6 (IL-6) as the main mediator, causing binding Signal Transducer and Activator of Transcription3 (STAT3) to the hepcidin promoter such that it increased its activity. Infusion IL-6 or inducing lipopolysaccharide increased levels of IL-6 in volunteers of healthy caused an increase in hepcidin followed by hypoferemia. Research in humans with chronic infections and inflammatory diseases weight also showed increased levels hepcidin which indicated that an increase in hepcidin levels play an important rolein anemia due to inflammation and blockage of the reticuloendothelial.[16]

Based on the data, it showed that hepcidin levels played a role in infection and inflammation in various diseases. However, until now, there is no research found specifically in Indonesia comparing the levels of hepcidin in children infected with STH. This study aimed to compare the hepcidin levels in STH-infected and non-infected children.

METHODS

This was a comparative observational analytical study conducted in a cross-sectional method. This research was conducted in elementary schools in Sunggal District, Deli Serdang in May-October 2018. The population in this study were primary school students of 6 -14 years of age. Samples were selected from 7 elementary schools and selected based on inclusion criteria from 1st until 6th classes. Samples were taken using non-probality sampling techniques with a minimum number of samples chosen as many as 160. The inclusion criteria were school students age 6-14 years and who agreed to take part in the study. Each research subject was given two plastic containers that had been labeled to accommodate feces and urine to examine the eggs of STH and hepcidin levels.

The stools of the subjects that had been put in plastic containers were collected and then examined to identify the species of STH in the Parasitology Laboratory of the Faculty of Medicine, Universitas Sumatera Utara. Forty milligrams of feces were examined using a microscope with the Kato Katz method to identify STH species and determine the degree of infection. The degree of infection was determined by the number of eggs/gram of stool (NEPG) as shown in Table 1. Harada-Mori's

Table 1. Degree of infection soil-transmitted helminths

Species	Degree of Infection		
	Mild	Moderate	Severe
Ascaris lumbricoides	< 7,000 epg	7,000-35,000 epg	>35,000 epg
Trichuris trichiura	< 5,000 epg	5,000-25,000 epg	>25,000 epg
Hookworm	< 2,000 epg	2,000-7,000epg	>7,000 epg

modification method was used to culture worm eggs in feces to identify hookworm species from their larvae. The results were classified according to the helminth eggs or hookworm larvae found in fecal cultures. In mixed infections, the degree of infection depended on which worms were more dominant.

The urine sample of the subject was collected and taken as much as 10 mL, then put into a centrifugation tube, then centrifugated at 2,500 rpm for 20 minutes. Then the supernatant was separated and put in aliquot as much as 1 mL. The urine sample was then stored at -20°C (could last ± 1 month). When analyzed, the sample flowed at room temperature about 30 minutes before the analysis was carried out. After that, the urine was processed and measured for hepcidin levels using Hepcidin Finetest® with the Sandwich-ELISA method in the Clinical Pathology Laboratory of the Haji Adam Malik Hospital. Measurements were made using a Spectrophotometer BD FACS Calibur with a wave length of 450 nm. The data obtained were processed using the SPSS. Data were tested for normality to determine the type of distribution. Univariate data were presented in a table and narrated. The comparison of hepcidin levels between STH-infected and non-infected children used the Wilcoxon test. The value of $p < 0.05$ was stated as significant.

This research was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sumatera Utara/Haji Adam Malik Hospital, with number 343/TGL/KEPK FK USU-RSUP HAM/2018.

RESULTS AND DISCUSSIONS

A total of 176 students had submitted stool samples, and only 168 of them had submitted urine samples. A total of 8 people were excluded from this study because they did not collect urine. The total number of stool and urine samples of students analyzed in this study were 168 samples

The characteristics of the samples assessed in this study included gender and age. Until the end of September 2018, of the 168 samples obtained in this study, those who met the study criteria consisted of 70 boys (41.67%) and 98 girls (58.33%). The median age of the sample was ten years. Of the 168 samples studied, 28 children were positively infected with soil-transmitted helminthes, as listed in Table 2.

Table 2 showed that the number of girls was more than boys in both groups. The number of girls in the STH-infected group was 16 (9.52%) and 82 in the non-STH infected group (48.80%). The number of boys in the STH-infected group was 12 (7.14%) and 58 (34.52%) in the non-infected group. Based on the description above, the gender distribution in the two groups was not so different. The average age of both STH-infected and the non-infected group was

Table 2. Characteristics of the subject in STH infected and non-infected

Characteristic	Positive STH (n= 28)	Negative STH (n= 140)	Total Sampel (n=168)	p
Gender				
Male	12(7.14)	58(34.52)	70(41.67)	
Female	16(9.52)	82(48.80)	98(58.33)	
Mean age	10.07±1.303	10.01±1.292		0.719
Type of infection				
Single infection	25(14.88)	0		
Mixed infection	3(1.78)	0		
Ascaris lumbricoides + *Trichuris trichiura*				
Helminths types				
Trichuris trichiura				
Ascaris lumbricoides	18(10.71)	0		
Hookworm	8(4.76) *	0		
Degree of infection	5(2.97)	0		
Mild				
Moderate	26(15.48)	0		0.978
Severe	1(0.59)	0		
Hepcidin (pg/mL)	1(0.59)	0		
	2686,14±922,21	2653,22 ± 926,09		

almost the same, in which the average age in STH-infected group was 10.07±1.303 years and in the non-infected group was 10.01±1.292 years, p=0.719. There were no differences in the chances for infection in boys and girls group, as also found by Elmi.[17]

Based on the stool examination of 168 study samples, the prevalence of STH infection was 16.66%. This result was not much different from that obtained by Fauzi *et al*., who found a prevalence rate of helminthiasis 20.7% from 58 samples obtained.[18] The prevalence of infection for each STH species was as follows: *Trichuris trichiura* 10.71%, *Ascaris lumbricoides* 4.76%, and hookworm 2.97%. Culture results from 5 positive samples infected with hookworm found 1 positive sample containing *Ancylostoma duodenale* species, and 4 more did not grow and develop. The results were also similar to a study by Fauzi *et al*., who found the most common type of helminth infection is *Trichuris trichiura*.[18] This result also corresponds to the Elmi *et al*. that from 542 samples, there are 440 children (81.3%) who suffered from *Trichuris trichiura*.[17] Based on the number of STH species that infect in this study, the prevalence of a single infection was 14.88% (25 cases) and mixed infections 1.78% (3 cases). Mixed infection is an STH infection of more than 1 species. In this study found 3 cases of mix infection. All three cases were found on the feces of eggs *Trichuris trichiura* and A*scaris lumbricoides.* The existence of a case of mix infection causes the total number of STH infections per species to increase 3 as shown in Table 2.

According to Table 2, there were found 26 cases (15.48%) with mild infection rates, one case (0.59%) with moderate infection rates, and also one case (0.59%) with severe infection rates. This result is similar with Elmi *et al*. who found that the highest degree of STH worm infection was a mild degree of 73.1% of all infected.[17]

This study did not evaluate urinary hepcidin levels, because hepcidin levels in urine samples did not differ significantly from serum as stated by Ayoya.[14] In this study, were found that hepcidin levels in the infected and uninfected groups were not significantly different (p=0.978). This result may be due to a mild infection rate showed in Table 2. Minor infections from STH will not have a significant clinical impact, especially on laboratory examination parameters. According to Cruz *et al*. the low prevalence of STH infections can be influenced by improved environmental hygiene, and sanitation and easy access to anti helmintic drugs.[19] According to Cherian *et al*., an increase in hepcidin levels is a preventative measure so that parasites cannot use iron by encouraging a decrease in blood ferritin levels.[13] This study is the first study to assess that hepcidin levels are not affected by STH infections at the degree of mild infections because these infections do not involve the role of macrophages and an increase in IL-6.

A limitation of this study was that researchers did not include inflammatory markers, such as Ferritin, CRP, and IL-6 which could help show the role inflammatory process that occurs in STH infections.

CONCLUSIONS AND SUGGESTION

There were no different hepcidin levels in children with and without soil-transmitted helminths infection. Further research is needed to obtain other parameters that are believed to have strong links such as cytokines or other pro-inflammatory agents.

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