Correlation between iron deficiency anemia and intestinal parasitic infection in school-age children in Medan

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Abstract. Anemia is an abnormal hemoglobin concentration in blood that impacts almost 40% school-age children in developing countries. Intestinal parasitic infection, along with malnutrition are contributed to influence absorption, transportation, and metabolism of iron which is the most common etiology of anemia in school-age children. The purpose of this study was to determine whether there is a correlation between iron deficiency anemia (IDA) and parasitic intestinal infection generally and protozoa infection particularly among school-age children in Medan. This was a cross-sectional study conducted from May until October 2016 in primary school in Medan and Hamparan Perak, Deli Serdang. Consecutive sampling was used with total 132 samples obtained. Univariate analysis and Bivariate analysis were performed. This study showed the prevalence of IDA was 7.6%, and proportion of parasitic intestinal infection was 26.5% with 19.8% protozoa infection. The correlation between IDA and intestinal parasitic infection was not significant in Chi-Square Test (p-value: 0.089), neither was between IDA and protozoa infection (p-value: 0.287). There was a correlation between MCV, MCH, and anemia with p-value<0.05. However, there was no correlation among other IDA variables such as Serum Iron, TIBC, ferritin, related to age, anemia, parasitic infection, and protozoa infection (p-value>0.05).

1. Introduction
Anemia is a silent disease that impacts almost 40% school-age children in developing countries.[1] Anemia is abnormal hemoglobin concentration in blood resulted from many factors, which is the most common cause is iron deficiency.[2] Factors contributing to this condition are increasing energy expenditure, irregularly eating habit, lacking maternal attention, and infection in particularly parasitic infections that common in school-age children.[3]

Intestinal parasitic infections are one of 17th neglected tropical disease listed by WHO and being the 4th top leading cause of communicable disease with a high percentage of disability.[4] The most prevalent parasites are helminths such as Ascaris lumbricoides, Hookworm, Trichuris trichiura,
Strongyloides stercoralis and protozoa such as Giardia lamblia, Entamoeba coli, Entamoeba histolytica, Iodamoeba butschlii.[2,5] The helminthic infection causes anemia deficiency by reducing iron uptake from the intestine, directly sucking blood, and interfering directly and indirectly in iron metabolism.[5] Protozoa impact anemia by destroying the intestine mucosal structure that influences in micronutrients absorption, such as iron. All these mechanisms also affect hosts’ nutritional status and then alter their immune system.[2] This condition becomes a vicious circle that impacts the quality of life, particularly in school-age children who still being in the golden age for developing body structure.[6]

Based on the studies above, the authors interest in examining the correlation between iron deficiency anemia (IDA) and parasitic intestinal infection among school-age children in Medan. The aim was to determine whether there is a correlation between iron deficiency anemia and parasitic intestinal infection generally and protozoa infection particularly at school age school in Medan.

2. Method
This research was an analytical cross-sectional study conducted from May until October 2016 in Public Primary School 060925 Harjosari 1, Medan Amplas and Public Primary School 101747 Hamparan Perak, Deli Serdang. The population in this study was primary school students in these schools. Sampling was carried out by consecutive sampling with 132 participants who matched inclusion criteria and had no exclusion criteria. The inclusion criteria were primary school grade III to VI aged 8-12 years, who were willing and had their parents’ approval to participate in this study. They also must not take anti-helminth or anti-protozoa medication in previous six months and had no history of asthma, atopic dermatitis, immunodeficiency, malignancies, rheumatic disease, and other parasitic infections. Exclusion criteria were stool and blood samples that were damaged or missing.

This study protocol was approved by Ethics Committee of Faculty of Medicine in Universitas Sumatera Utara. The authors also took the permission from primary school authority.

Data was collected by examining the stools and peripheral blood smears. After participants and their parents had understood the contents of the study and filling informed consent, the researchers gave form of characteristic, history of the previous disease, and drug consumption of the participants. They were also given a stool pot to collect feces in the morning. Feces preserved in 10% formalin for transporting parasites to a laboratory where it stained and examined under the microscope. Stool examination using Kato-Katz technique. Diagnosis confirmed by finding eggs or larvae of helminths and trophozoites or cysts of protozoa. When participants collected their stool pot, peripheral blood smears carried out for establishing the diagnosis of anemia and morphology of erythrocytes. About four mL blood samples were taken using a venipuncture technique, collected in a tube containing EDTA for anticoagulant, and then reserved in a cold storage box and transported back to the hematology laboratory. All blood samples analyzed less than 10 hours after blood collection. Hemoglobin level, MCH, and MCV were measured using SYSMEX T-2000i automatic hematology analyzer. Serum iron, ferritin, and TIBC were measured by COBAS 6000 e 501.

All the collected data was entered and analyzed by using PASW (Predictive Analytics Software) for Mac version 20. Data was described in the distribution of frequencies then analyzed using Chi-Square ($\chi^2$) for bivariate analysis to know whether there is a correlation between IDA and parasitic infection and between IDA and protozoa infection. Then, all data variables such as serum iron, ferritin, MCH, MCV, and TIBC were analyzed using ANOVA to determine whether there is any statistically significant difference among them according to intestinal parasitic infection and protozoa infection. The test considered significant with p-value< 0.05 (CI 95%).

3. Results
Total 132 students aged 8-12 years old from grade 3,4,5,6 Public Primary School Harjosari 1, Medan Amplas and Hamparan Perak, Deli Serdang enrolled in this study. Among them, there were 54.5% boys and 45.5% girls.
Table 1. General characteristics of school-age children conducted in study.

| Characteristics                             | N/%   (n=132) |
|---------------------------------------------|-----------|
| Gender                                      |           |
| • Boys                                      | 72 / 54.5%|
| • Girls                                     | 60 / 45.5%|
| Parasitic Infection                         |           |
| • Yes                                       | 35 / 26.5%|
| • No                                        | 97 / 74.2%|
| Protozoa Infections                         |           |
| • Yes                                       | 25 / 18.9%|
| • No                                        | 107 / 81.1%|
| Type of Parasites Infection                 |           |
| • Entamoeba coli                            | 8 / 6%    |
| • Giardia lamblia                           | 6 / 4.5%  |
| • Ascaris lumbricoides                      | 4 / 3%    |
| • Hookworm                                  | 1 / 0.8%  |
| • Trichuris trichiura                       | 5 / 3.8%  |
| • Iodamoeba butchlii                        | 2 / 1.5%  |
| • E. coli and G. lamblia                    | 7 / 5.3%  |
| • G. lamblia and I. butchlii                | 1 / 0.8%  |
| • E. coli and I. butchlii                   | 1 / 0.8%  |
| Anemia (Hb< 11.5 gr/dL)                     |           |
| • Yes                                       | 10 / 7.6% |
| • No                                        | 122 / 92.4%|
| Serum Iron (low < 50 µg/mL)                 |           |
| • Low                                       | 15 / 11.4%|
| • Normal                                    | 117 / 88.6%|
| Ferritin (Low < 12 µg/mL)                   |           |
| • Low                                       | 0 / 0%    |
| • Normal                                    | 100 / 100%|
| TIBC (High TIBC > 346 µg/dL)               |           |
| • High                                      | 55 / 41.7%|
| • Normal                                    | 77 / 58.3%|
| MCV (78-98 fl)                              |           |
| • Microcytic                                | 59 / 44.7%|
| • Normocytic                                | 73 / 55.3%|
| • Macrocytic                                | 0 / 0%    |
| MCH (25-33 pg)                              |           |
| • Hypochromic                               | 16 / 12.1%|
| • Normochromic                              | 116 / 87.9%|

In this study, there were 34 students (25.8%) infected by parasites, with 18.9% protozoa infections and 6.9% STH infections. The most prevalent were infected by Entamoeba coli (6%). There were 10 students (7.5%) considered anemia (Hb<11.5 gr/dl) with 11.3% in low serum iron levels, 41.4% high TIBC, 45.2% microcytic erythrocytes, and 12.1% hypochromic erythrocytes.

Table 2. Comparison and bivariate analysis of iron deficiency anemia (IDA) variables in infected and noninfected parasitic group, infected and noninfected protozoa group.

| Parasitic Infection | Noninfected | Infected | P value | Protozoa Infection | Noninfected | Infected | P value |
|---------------------|-------------|----------|---------|-------------------|-------------|----------|---------|
| Anemia Yes          | 5 / 5.1%    | 5 / 3.5% | 0.089   | 7 / 6.5%          | 3 / 12%     | 0.287    |
| Anemia No           | 92 / 94.9%  | 30 / 85.8%| 0.060   | 100 / 93.5%       | 22 / 88%    | 0.428    |
| Serum Iron Low      | 11 / 11.3%  | 4 / 11.4% | 0.602   | 13 / 12.1%        | 2 / 8%      | 0.428    |
Anemia is a global health problem that affects many people in each socioeconomic status, age, and sex all over the world. In 2011, WHO estimates about 800 million children and women suffered anemia.[7] In 2013, the proportion of anemia in Indonesia was approximately 21.7% as general and 26.4% in school-age children.[8] In our study, the prevalence of anemia among school-age children was about 7.6%. It is lower than prevalence anemia worldwide and in Indonesia. It is probably caused by improving the nutritional status of school-age children so that their immune system can withstand the parasite and prevent to develop the disease.

Several intestinal protozoa and soil-transmitted helminths are classified as the neglected tropical disease that affects more than 1 billion people around the world. Worldwide, there are 149 countries affected one of neglected tropical disease and often, they are suffered from more than one infection.[9] Like in this study, 9 of 34 children got more than one parasitic infections. Mostly, they were infected by intestinal protozoa and soil-transmitted helminths one time because of the same route of disease, such as waterborne and foodborne. In this study, the most common parasitic infections were caused by E. coli (6%) and G. lamblia (4.5%), and the most common helminths infection were caused by T. trichiura (3.8%) and A. lumbricoides (3%). This study was almost similar to Galvao’s study in Brazil that found that most prevalent parasitic infections are E. coli (27.46%), and G. lamblia (26.49%).[2] In another study in South Africa, Nxsana found the most prevalent parasitic infections are A. lumbricoides (29%), G. lamblia (9.9%), Entamoeba sp (6.8%).[10]

There are many factors contribute to anemia, but the most prevalent are iron deficiency anemia, around 50% cases.[7] In developing countries, parasitic infections are the most common cause of iron deficiency anemia, particularly in children. Parasites can invade human and cause anemia in few ways. Hookworm caused intestinal blood loss through mechanical rupture of blood vessel in the intestine then leading to iron deficiency and protein malnutrition. This worm also secretes some anti-

| level | Normal | Microcytic | Normocytic | 0.049 | 0.858 | 0.254 | 0.000 | 0.000 |
|-------|--------|------------|------------|-------|--------|--------|--------|--------|
| MCV   | 86/88.7% | 48/49.4%  | 49/50.5%   |       |         |        |        |        |
| MCH   | 13/13.4%  | 11/31.4%  | 24/68.6%   | 0.115 | 0.379  |        |        |        |
| TIBC  | 11/31.4%  | 24/68.6%  | 84/86.6%   | 0.339 | 14/31. %| 44/41.1%| 44/41.1%| 44/41.1%|

From table 2, we can see the cross tabulation among anemia, serum iron level, TIBC, MCV, and MCH compared to parasitic infection and protozoa infection. There was a significant correlation between MCV and intestinal parasitic infection with p-value < 0.05, although there was no significant correlation between anemia and other IDA variables such as serum iron, TIBC, and MCH (p-value > 0.05).

### Table 3. p-value of each IDA variables analyzed using one way analysis of varian (ANOVA).

| Age  | Anemia | Parasitic Infection | Protozoa Infection |
|------|--------|---------------------|--------------------|
| Serum Iron | .658 | .257 | .858 | .287 |
| TIBC | .654 | .383 | .254 | .727 |
| Ferritin | .367 | .721 | .733 | .253 |
| MCV | .781 | .000 | .911 | .758 |
| MCH | .876 | .000 | .914 | .854 |

Table 3 presented the p-value of one-way ANOVA results to compare mean differences of serum iron, TIBC, ferritin, MCV and MCH according to age, anemia status, parasitic infection, and protozoa infection. It showed that there was a correlation between MCV, MCH, and anemia with p-value<0.05. However, there was no correlation among other IDA variables such as Serum Iron, TIBC, ferritin, related to age, anemia, parasitic infection, and protozoa infection (p-value>0.05).

### 4. Discussion

Anemia is the global health problem that affects many people in each socioeconomic status, age, and sex all over the world. In 2011, WHO estimates about 800 million children and women suffered anemia.[7] In 2013, the proportion of anemia in Indonesia was approximately 21.7% as general and 26.4% in school-age children.[8] In our study, the prevalence of anemia among school-age children was about 7.6%. It is lower than prevalence anemia worldwide and in Indonesia. It is probably caused by improving the nutritional status of school-age children in these school so that their immune system can withstand the parasite and prevent to develop the disease.
coagulant and antiplatelet agents that increase the amount of the blood loss.[11] *T. trichiura* even invades directly into the large intestine and causes blood loss. While attacking the mucous, it also sucks micronutrients from the intestine. Usually, people with trichiuriiasis infection has anorexia which can decrease intake of nutrition. These processes lead to iron deficiency anemia.[2] *Ascaris lumbricoïdes* may impair micronutrient absorption such as iron in duodenum and jejenum that leads to iron deficiency anemia.[12] Protozoa infection has its other ways; it interacts to small intestine mucous that make villous atrophy in various degree, along with causing inflammamatory infiltrate and crypt hypertrophy. These processes break the enterocytes and alter bile acid metabolism that impacts to poor absorption of macro and micronutrient essential for body function, such as vitamin, iron, zinc, and folic acid.[2]

In this present study, children with parasitic and protozoa infections mainly had the higher percentage of iron deficiency anemia (IDA) than noninfected group although it did not correlate significantly in statistical analysis. Few studies confirmed that there was a correlation between intestinal parasite and anemia, like Galvao’s retrospective study in Brazil. He found that there was a strong correlation between *Giardia lamblia, Trichuris trichiura*, *Strongyloides stercoralis* and anemia in 302 cases of intestinal parasites in every age in Araquara Hospital Brazil.[2] In Southeast Asia, Le Huong Thi also showed that there was significant correlation between parasitic intestinal infection, particularly *T. trichiura* and iron deficiency anemia in school children in Rural Vietnam.[13] In contrast, in another study in Brazil found there was no correlation between anemia and parasitic intestinal infection among primary school children.[14]

There are few variables of iron deficiency anemia such as Hemoglobin (Hb), MCV, MCH, serum iron, ferritin, and TIBC that we can associate with parasitic infection and protozoa infection. In this study, we could not show any meaningful differences among them correlate to parasitic infection and protozoa infection in Anova analysis. It was contrast to Galvao study in Brazil that stated there was an association among Hb, MCV, MCH, and erythrocyte account compared to parasitic intestinal infection.[2] However, in another study in Alagoas, Brazil, Silva could not find the association among Hb, ferritin, and serum iron compare to parasitic intestinal infection.[15] These finding probably caused by the nutritional improvement of school-age children. They were not anemia because they had enough nutrition and immune system to decrease the impact of parasitic infection.

5. Conclusion
There was no significant correlation between IDA and parasitic intestinal infections; neither was between IDA and protozoa infection in school-age children in Medan. The prevalence of parasitic intestinal infection was still high, so we recommend to every primary school and parents to pay more attention to sanitation and personal hygiene of school-age children to prevent spreading and developing of intestinal parasitic agents.

References
[1] Assefa S, Mossie A and Hamza L 2014 Prevalence and severity of anemia among school children in Jimma Town Southwest Ethiopia *Bio. Med. Central Hematol.* 14(3)
[2] Carrilho G F, DaCosta G M, Olivi M J, *et al.* 2011 Anemia in patients with intestinal parasitic infections *Parasitol.* 70(6) 206-11
[3] Akeredolu I A, Oguntona G E, Ocofor C, *et al.* 2011 Iron, zinc, and copper malnutrition among primary school children in Lagos, Nigeria *Scientific Res.: Food Nutr. Sci.* (2) 1063-70
[4] Trebi and Nicholas I N 2017 Emerging and neglected infectious diseases: Insights, advances, and challenges *Bio. Med. Res. Int.* Article ID 5245021 Available from: https://doi.org/10.1155/2017/5245021
[5] Adebara O V, Ernest S K and Ojuawo I A 2011 Association between intestinal helminthiasis and serum ferritin levels among school children *Scientific Res.: Open J. Pediatr.* (1) 12-6
[6] Chajhlana S P S, Mahabharat R N and Varaprasa M S M 2017 Nutritional deficiencies among school-age children in urban areas of Hyderabad Telangana India Int. J. Comm. Med. Pub. Health 4(2)

[7] WHO 2015 The global prevalence of anemia in 2011 (Geneva: World Health Organization)

[8] Kemenkes RI 2013 Riset kesehatan dasar (Riskesdas)

[9] Erismann S, Diagbouga S, Odermatt P, et al. 2016 Prevalence of intestinal parasitic infections and associated risk factors among school children in the Plateau Central and Centre-Ouest Regions of Burkina Faso Biomed. Centr.: Parasites and Vector (9) 554 Doi: 10.1186/s13071-016-1835-4

[10] Center for Disease Control and Prevention 2017 Parasites-neglected tropical disease (Atlanta: CDC)

[11] Nxasana N, Bhat V G and Vasikar S D 2013 Prevalence of intestinal parasites in primary school children of Mthatha, Eastern Cape Province, South Africa Ann. Med. Health Sci. Res. 3(4) 511-6 Doi:10.4103/2141-9248.122064

[12] Banu H, Khanum H and Hossain M A 2014 Relationships between anemia and parasitic infections in adolescent girls of Bangladesh Bangladesh J. 42(1) 91-103

[13] Osazuwa F, Ayo O M and Imade P A 2011 Significant association between intestinal helminth infection and anaemia burden in children in rural communities of Edo state, Nigeria North Am. J. Med. Sci. 3(1) 30-4

[14] Ngui R, Lim Y A L, Chong K L, et al. 2012 Association between anaemia, iron deficiency anaemia, neglected parasitic infections and socioeconomic factors in rural children of west Malaysia PLoS Negl. Trop. Dis. 6(3) e1550 doi:10.1371/journal.pntd.0001550

[15] da Silva J V L, Fontes G, dos Santos R V, et al. 2016 Factors associated with gastrointestinal parasitic infections among young population in Northeast Brazil Can. J. Infect. Dis. Med. Microbiol.