Original article:

Intestinal Parasitic Infections and Eosinophilia: A Cross-sectional Study among Primary School-aged Children in Medan, Indonesia

Muhammad F Rozi[1], Dewi M Darlan[2], Rodiah Rahmawati[3], Dewi IS Siregar[4]*

Abstract:

Background:Intestinal parasitic infections (IPIs) anditsimplication, such as malnutrition, growth stunting, anemia, and concentration impairment, still become a global burden. The primary immune cell that firstly involved in counteracting parasitic invasion is eosinophil. Therefore, higher levels of eosinophils could be suspected of havinga parasitic infection. ***Objective:*** Our study aimed to revealthe prevalence of IPIs and its correlation with eosinophilia.***Material and Methods:*** The study was located in two different public primary schools,Public Primary School 060925 Harjosari 1, Amplas, Medan, Indonesia and Public Primary School 101747 Hamparan Perak, Deli Serdang, Indonesia which enrolled 132 primary school children aged 8-12 years graded III-VI, consisting of 22 males and 110 females,who had met the inclusion criteria between May and October 2016. Parasitology examination was carried out at the Department of Parasitology, Faculty of Medicine, Universitas Sumatera Utara using Kato-Katz, Lugol, trichrome, and modified acid-fast stain.***Results:*** The study found intestinal protozoa infections were the most common IPIs in the population, *Giardia lamblia* as the most prevalent species (37.1%), while hookworm with the fewest findings (2.8%). Additionally, the statistical analysis proved a significant correlation between IPIs and eosinophilia (*p-value* 0.021; 95% CI 1.13-5.58). ***Conclusion:*** eosinophilia patients with profound clinical manifestation should be further assessed to be considered for the administration of anti-parasitic medication.

Keywords: Parasites, helminthiasis, eosinophils, protozoa

International Journal of Human and Health Sciences Vol. 04 No. 04 October'20 Page : 277-281
DOI: http://dx.doi.org/10.31344/ijhhs.v4i4.213

Introduction

Intestinal parasitic infections (IPIs) are parasitic infections caused by helminthic and protozoal manifestations, and it remains a neglected health problem for developing countries, particularly Indonesia. IPIs could clinically produce mild diarrhea in humans or severe debilitating symptoms and long-term complications. Chronic infection of the organism frequently leads to malnutrition, growth stunting, and cognitive impairment among children.[1]IPIs are separated into two different groups, Soil-transmitted Helminth (STHs) and intestinal protozoa infections, based on its etiologic agent. *Giardia lamblia*, *Cryptosporidium parvum*, and *Entamoeba histolytica* are the most common intestinal protozoa infection. *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworm are the most prevalent STH. The prevalence of IPIs is varied, higher in developing regions ranging from 34.2- 65.5 % in Ethiopia[2,3], 31 % in Indonesia[4], and 65-90.4 % in Sudan.[5,6]

The initiation of immune response starts as early

1. Faculty of Medicine, Universitas Sumatera Utara, Jl. dr. Mansur Kampus USU Medan, Indonesia
2. Parasitology Department, Faculty of Medicine, Universitas Sumatera Utara, Jl. dr.Mansur Kampus USU Medan, Indonesia
3. Opthalmology Department, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
4. Clinical Pathology Department, Faculty of Medicine, Universitas Sumatera Utara, Medan, North Sumatera, Indonesia

Correspondence to: Dewi Masyithah Darlan, Jln. Dr. T. Mansur, Kampus USU Padang Bulan, Medan 20155, Indonesia, Department of Parasitology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia. Email: dmasyithah57@gmail.com, tel: +628111644545

as the organism firstly invaded the outer barrier of the intestinal mucosa. The recognition and effector immune cell spends for at least hours or days, but eosinophil acts a pivotal role in primary response for parasitic invasion concurrent with the other active immune reaction. Eosinophil counts for 2-3% of the total leukocytes in human; it will fully function after the exposure of specific virulence factor followed by a cytotoxic effect in the mucosal environment. Therefore, It will elevate or so-called 'eosinophilia' as a result of parasitic invasion or emerging autoimmune disease. Neglecting eosinophilia patients could cause significant implication towards the patient quality of life. There must be a thorough anamnesis and precise information relating to existing risk factors and previous illnesses which supported by physical and laboratory examination.[7]

The major inducer for eosinophil proliferation is interleukin-5 (IL-5), which produced as aresponse of parasitic infections. IL-5 plays an essential role as the eosinophil activator after its secretion from the activated T-helper 2 (Th2) which exposed to intestinal parasites.[8] The primary objective of the study was to identify the species of human intestinal parasitic infections (IPIs)among primary school-aged children and assessed the correlation between the prevalence of IPIs andeosinophilia. The findings of the study would strengthen the evidence of the association between eosinophilia and parasitic intestinal infections that help the clinician to determine the diagnosis.

Materials and Methods

Study location and population

We conducted the cross-sectional study in Medan, Indonesia which is located along the northeastern coast of Sumatera Island with a coordinate of 3°35′N 98°40′E. There were approximately 2,097,610 people in the city based on the 2010 census. The regional climate is known as tropical rainforest climate with no significant dry season and average temperatures 27^0C (81^0F) throughout the year which is very supportive for the parasitic proliferation and development.

There were 132 school-aged children, 22 males and 110 females, from two different public primary schools, Public Primary School 060925 Harjosari 1, Medan and Public Primary School 101747 Hamparan Perak, Deli Serdang, Indonesia who enrolled into the study between May and October 2016. The study location was selected as the matter of its densely populated characteristic with poor hygiene and sanitation environment. The guardian's consent is the main inclusion criteria for the children while the other obligatory criteria, includingaged 8-12 years, graded III-VI, no consumption of anti-helminthic drug for the past six months, and no history of immunological disorder (allergy, asthma, atopic dermatitis, rheumatic disease, immunodeficiency, malignancies, other infectious disease or hemophilia).

Figure 1. The northern part of Sumatera Island (Black marker: Medan).

There was a brief oral explanation to all guardians who gave permissions for the children enrollment of the study as well as filling the short questionnaire for any previous or existing illnesses. One small plastic fecal containerlabeled with the children's name was given to the guardian for the fecal sample examination in the Department of Parasitology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Parasitological examination and eosinophil count

The parasitological examination for helminthic and protozoal species includes Kato-Katz, as its sensitivity reached 74-95%when used in places with high infection rate[9],Lugol, trichrome staining, and modified acid-fast staining or Kinyoun-gabbet for *Cryptosporidium sp.* infections[10]. In addition, eosinophil count was based on traditional technique using an improved Neubauer counting method and peripheral blood smear,calculating the absolute value in 1 mm^3 per high-power field, under a light microscope. Blood was obtained from venous sampling via venipuncture to cubital vein as much as 3 ml which directly mixed in a tube containing EDTA. The interpretation for eosinophil count has two different categories, normal (1-6 %) and eosinophilia (>6%).

Data Analysis and study approval

All data were doubled-checked in Microsoft Excel before the final analysis using Statistical Package for Social Sciences 21 (SPSS Inc. version 21).

Subsequently, the correlation between eosinophilia and IPIs were demonstrated using p-value < 0.05 which extracted fromthe Spearman correlation test and presented the data into a single table. The study has also obtained approval from the Medical Ethics Committee, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia.

Results

A total of 35 children were positive for IPIs consisting of 10 samples (28.5%) forSoil-transmitted helminths (STHs) and25 samples (71.5%) for intestinal protozoa infections. The variation was demonstrated in Table 1 with *Giardia lamblia* as the prevalent species followed by *Entamoeba coli;* it showed that protozoal infection still predominated the infected children in the study. The prevalence of intestinal protozoa infections outnumbered STH findings among the population (71.4% versus 28.6%). Meanwhile, eosinophils were calculated under routine microscopic examination and produced the results demonstrated in Table 1 and Table 2. The study also obtained a significant correlation between IPIs and eosinophilia (*p-value*=0.021) and the higher median value of eosinophil levels among IPI-infected children was evident.

Tabel 1. Intestinal Parasitic Infection (IPI) species and effect on eosinophil levels

IPIs species	n=35	%	Eosinophil levels (Median±SD)
Ascaris lumbricoides	4	11.4	6.05 ± 2.21
Hookworm	1	2.8	2.5± 0.00
Trichuris trichiura	5	14.2	6.7 ± 3.2
Giardia lamblia	13	37.1	4.7 ± 7.14
Entamoeba coli	8	22.8	3.7 ± 2.82
Iodoamoebabutschii	3	8.5	3.1 ± 1.78
G.lamblia&I.butschii	1	2.8	2.8± 0.00

Table 2. Correlation between Intestinal Parasitic Infections (IPIs) and eosinophil levels among school-aged children

Variable	n=132	%	Eosinophil levels (Median±SD)	r	p-value	PR (95% CI)
IPIs (n=132)						
Yes	35	26.5	4.50 ± 5.36	0.197	0.021	2.51 (1.13-5.58)
No	97	73.5	3.60 ± 3.41			

Discussions

The study result demonstrated the infection rate for 26.5% of 135 children which proved on findings of the parasitological examination. They were positive for seven distinct parasitological species with specific pathogenesis and virulence factors. The high-prevalent of IPIs among children gave the evidence that the infection remainsa neglected tropical infectious disease among population whereas children are significantly affected by the infection. Several implications related to IPIs were emphasized from recent studies particularly in densely populated areas and associated with poor sanitation, inadequate access to clean water,lack of proper hygiene and sanitation knowledge.[11]Poverty can also lead to an increase in the prevalence of IPIs as well aslow socioeconomic statusthat vulnerably suffer from a vicious circle of reinfection, leading to the higher morbidity.In the previous study, it was found that 40% ofchildren in a similar location infected with STH and more than one-third of children were categorized as underweight. Underweight is noticeable as one of the clinical implications relating to chronic parasitic infection because it affects micronutrient absorption and ultimately increases the prevalence of malnutrition in the infected population.[12]

Our study showed that intestinal protozoa infections are prevalent among children. *Giardia lamblia* appeared as the frequent infections of all IPIs (37.1 %) while the second most frequent was *Entamoeba coli* (22.8%). Similarly, Mehraj et al. and Chaudhry et al. conducted two different studies in Pakistan discovered that intestinal protozoa infection were highly prevalent, while *Giardia lamblia* became the main species found in the survey study.[13,14]*Giardia lamblia* (also known as *G.duodenalis* and *G.intestinalis*) is a common intestinal protozoal species which infected 280 million people each year worldwide.[15]*G.lamblia* has potential to cause persistent and recurrent infections as it is ubiquitous organism which also related to several clinical implications including worsening bowel permeability, growth impairment, wasting, and cognitive impairment.[16]

Meanwhile, STH infections are also strongly associated with poor knowledge of proper behavior related to defecation process, hand washing, and the exposure with infected soil with fertilized eggs and larvae.[17]Additionally,the infection burden is also highly associated with the environment as well as become a modifiable risk factor causing IPIs. Behavior, genetically susceptible, inadequate health care, and malnutrition are also increase the susceptibility of an individual infected with IPIs.[18]Several studies conducted in different locations suggested that school-aged children

suffered the most from various complications of the infection, such assignificant iron deficiency anemia which may lead to decreased concentration, physical and cognitive development that are finally reducing school performance.[19]

Blood component builds human immune system to serve as natural resources in eradicating foreign antigen invasion, including lymphocytes, neutrophils, monocyte, and eosinophils. Eosinophilia can occur as a result of two pathological conditions, infectious and non-infectious process that is hard to distinguish each of two clinical entities. The clinician should consider the type of patient, concurrent symptoms, eosinophilia duration, and the severity of eosinophilia to strengthen the clinical evidence relating to the suspected diagnosis.[7]Acute presentation of eosinophilia could represent autoimmune disease, acute schistosomiasis, or coccidiomycosis infection. Helminthiasis also appears with eosinophilia, notably coinciding with larval migration through tissue.

Based on our study,there was asignificant correlation between IPIs and eosinophilia (*p-value*< 0.021). Ustun et al. also foundsimilar result suggesting that eosinophil levels were higher among IPIs than in the control group (7.0 % versus 6.5%) but not statistically significant.[20] The study also observed higher levels of IL-5 in the group which was positive for a protozoal infection and statistically significant proven (*p-value*<0.05). Kaminsky et al. conducted a study to observe IPIs and eosinophilia among HIV positive population; they found that STHs were highly prevalent among the people and correlated with eosinophilia (*p-value*< 0.05).[21]Al-Mozan et al. examined the alteration of the blood component in IPIs patients, and concluded the findings ranges from the increase and decrease of each blood component still following the particular parasitic species.[22] Higher levels of eosinophils emerged in mixed infection (6%) as well as*Hymenolepsis nana* and protozoa infections.In our study, it was found that single infection of soil-transmitted helminths (STHs), such as*T.trichiura* and *A.lumbricoides* samples, had higher eosinophils levels (median 6.7% and 6.05% respectively) compared to other IPIs and the lowest was found in hookworm and mixed infection (*G.lamblia* and *I.butschii*), with the median of 2.5% and 2.8%.

Conclusions

Intestinal Parasitic Infections (IPIs) are described as one of neglected tropical infectious disease as it is relentlessly producing clinical implications in the general population, mainly in school-aged children. The study findings could add evidence of eosniophilia individuals who suffered from a parasitic infection that directly could help the clinician to narrow the differential diagnosis. Furthermore, the presence of eosinophilia among high-risk patients will lead to further examination, including fecal examination, or eventhe administration of antihelminthic and antiprotozoal medications. Additionally, further study is also needed to determine the level of eosinophil threshold that can be used as a marker of infection with larger sample size.

Conflict of interest declaration: There was no conflict of interest

Ethical clearance: The study has been approve by the ethical medical research committee, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Author's contribution: First and Second author has involved in whole stages of publication process while the rest contributed for data analysis until manuscript preparation.

References:

1. Di Genova BM, Tonelli RR. Infection strategies of intestinal parasite pathogens and host cell responses. Frontiers in microbiology. 2016 Mar 3;7:256.
2. Gelaw A, Anagaw B, Nigussie B, Silesh B, Yirga A, Alem M, Endris M, Gelaw B. Prevalence of intestinal parasitic infections and risk factors among schoolchildren at the University of Gondar Community School, Northwest Ethiopia: a cross-sectional study. BMC public health. 2013 Dec;13(1):304.
3. Hailegebriel T. Prevalence of intestinal parasitic infections and associated risk factors among students at Dona Berber primary school, Bahir Dar, Ethiopia. BMC infectious diseases. 2017 Dec;17(1):362.
4. Uga S, Kimura D, Kimura K, Margono SS. Intestinal parasitic infections in Bekasi district, West Java, Indonesia and a comparison of the infection rates determined by different techniques for fecal examination. Southeast Asian journal of tropical medicine and public health. 2002 Sep;33(3):462-7.
5. Siddig HS, Mohammed IA, Mohammed MN, Bashir AM. Prevalence of intestinal parasites among selected group of primary school children in Alhag Yousif Area, Khartoum, Sudan. Int J Med Res Health Sci. 2017 Jan 1;6(8):125-31.
6. Al-Mohammed HI, Amin TT, Aboulmagd E, Hablus HR, Zaza BO. Prevalence of intestinal parasitic infections and its relationship with socio–demographics and hygienic habits among male primary schoolchildren in Al–Ahsa, Saudi Arabia. Asian Pacific Journal of Tropical Medicine. 2010 Nov 1;3(11):906-12.
7. O'Connell EM, Nutman TB. Eosinophilia in infectious diseases. Immunology and allergy clinics of North America. 2015 Aug;35(3):493.
8. Huang L, Appleton JA. Eosinophils in helminth infection: defenders and dupes. Trends in parasitology. 2016 Oct 1;32(10):798-807.
9. Nikolay B, Brooker SJ, Pullan RL. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: a meta-analysis in the absence of a true gold standard. International journal for parasitology. 2014 Oct 1;44(11):765-74.
10. McHardy IH, Wu M, Shimizu-Cohen R, Couturier MR, Humphries RM. Detection of intestinal protozoa in the clinical laboratory. Journal of clinical microbiology. 2014 Mar 1;52(3):712-20.
11. Ngui R, Ishak S, Chuen CS, Mahmud R, Lim YA. Prevalence and risk factors of intestinal parasitism in rural and remote West Malaysia. PLoS Neglected Tropical Diseases. 2011 Mar 1;5(3):e974.
12. Darlan DM, Alexandra T, Tala Z. Soil Transmitted Helminth Infections in Medan: a cross--sectional study of the correlation between the infection and nutritional status among elementary school children. Family Medicine & Primary Care Review. 2017(2):98-103.
13. Mehraj V, Hatcher J, Akhtar S, Rafique G, Beg MA. Prevalence and factors associated with intestinal parasitic infection among children in an urban slum of Karachi. PloS one. 2008 Nov 10;3(11):e3680.
14. Chaudhry ZH, Afzal M, Malik MA. Epidemiological factors affecting prevalence of intestinal parasites in children of Muzaffarabad district. Pakistan J. Zool. 2004;36(4):267-71.
15. Bartelt LA, Sartor RB. Advances in understanding Giardia: determinants and mechanisms of chronic sequelae. F1000prime reports. 2015;7.
16. Al-Mekhlafi HM, Al-Maktari MT, Jani R, Ahmed A, Anuar TS, Moktar N, Mahdy MA, Lim YA, Mahmud R, Surin J. Burden of Giardia duodenalis infection and its adverse effects on growth of schoolchildren in rural Malaysia. PLoS neglected tropical diseases. 2013 Oct 31;7(10):e2516.
17. Ziegelbauer K, Speich B, Mäusezahl D, Bos R, Keiser J, Utzinger J. Effect of sanitation on soil-transmitted helminth infection: systematic review and meta-analysis. PLoS medicine. 2012 Jan 24;9(1):e1001162.
18. Yap P, Utzinger J, Hattendorf J, Steinmann P. Influence of nutrition on infection and re-infection with soil-transmitted helminths: a systematic review. Parasites & vectors. 2014 Dec;7(1):229.
19. Brooker S. Estimating the global distribution and disease burden of intestinal nematode infections: adding up the numbers–a review. International journal for parasitology. 2010 Aug 15;40(10):1137-44.
20. Ustun S, Turgay N, Delibas SB, Ertabaklar H. Interleukin (IL) 5 levels and eosinophilia in patients with intestinal parasitic diseases. World Journal of Gastroenterology: WJG. 2004 Dec 15;10(24):3643.
21. Kaminsky RG, Soto RJ, Campa A, Baum MK. Intestinal parasitic infections and eosinophilia in an human immunedeficiency virus positive population in Honduras. Memórias do Instituto Oswaldo Cruz. 2004 Nov;99(7):773-8.
22. Al-Mozan HD, Daoud YT, Dakhil KM. Intestinal parasitic infection effect on some blood components. Journal of Contemporary Medical Sciences. 2017 Mar 26;3(9):159-62.