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Increased IL-4 and Ig E Level in Hookworm Infection in Plantation Workers in JEMBER

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Abstract

Hookworm infection is a disease that is included in the neglected tropical disease. Hookworm infection is suffered by many plantation workers who, in daily life are closely related to the soil. Immune response in hookworm-infected individuals will experience disorder especially in eosinophilia, mastocytosis and IgE stimulation. It is, therefore essential to know the immune response to hookworm infections in plantation workers. The study subjects in this study consisted of 25 hookworm-infected subjects and 25 healthy subjects. The study was conducted in November 2021 on plantation workers in 5 sub-districts in Jember. This study calculated eosinophilia on peripheral blood smears and the number of IL-4 and Ig-E levels using the Elabscience® ELISA kit with two repetitions. The results showed an increase in eosinophil levels from patient blood samples (average 5.43%), and a significant increase in IL-4 and IgE levels based on Mann Whitney's statistical analysis (p-value = 0.000). It can be concluded that IL-4 and IgE levels can be used as a marker of diagnosis of hookworm infection.

Keywords*: hookworm, plantation worker, IL-4, and IgE*

Introduction

Hookworm infection is a disease that is included in neglected tropical diseases (NTD (Hotez et al., 2008)). Hookworms are included in the soil-transmitted helminth (STH) group, which are worms that require soil media to develop into an infective form (Supali et al., 2017). Two species of hookworm can infect humans, namely *Ancylostoma duodenale* and *Necator americanus.* The prevalence of hookworm infection in the world is still high, 700 million people are infected and cause 3000 to 65000 mortality annually (Walana et al., 2014). Hookworm infection is the most important of the neglected tropical diseases in terms of causes morbidity, accounting for 3.2 million disability adjusted life years (DALYs) lost annually (Loukas et al., 2016). In Indonesia, the prevalence of hookworm ranging from 30% to 50% in various regions, particularly in region with poor higyene and sanitation (Tapiheru & Zain, 2021).

The occupational factor is one of the risk factors associated with hookworm infection. Jobs in agriculture and plantations have a high risk of hookworm infection. A previous study on plantation workers in Jember showed a prevalence of hookworm infection of 21.3% (Amanullah et al., 2021).This is because hookworm larvae can thrive in moist, loose, sandy soil and contain lots of compost, such as soil in plantation areas (Rusmartini et al., 2014).

The primary immune response disorders in individuals infected with hookworm include eosinophilia, mastocytosis, and IgE stimulation. Eosinophilia is an immune response that occurs early in acute infection. Mast cell degranulation happens in response to the allergen interaction of worms with IgE. Protease from mast cells will degrade collagen in the cuticular part of adult hookworms in the intestinal lumen. IgE antibodies play a role in the cellular degranulation of eosinophils, basophils, and mast cells and subsequently cause toxic activity for hookworms (Gazzinelli-Guimaraes & Nutman, 2018).

The heterogeneous immune response in individuals infected with hookworm affects the clinical course of this infectious disease. Previous studies have shown that the immune response is related to the intensity of infection and may provide some protection (Girgis et al., 2013). By knowing the increase in IL-4 and IgE levels, it will support the diagnosis of hookworm infection. Therefore, it is necessary to carry out further research on the immune response to hookworm infection in plantation workers as part of a strategy for controlling and preventing

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hookworm infection, especially in plantation areas.

Methods

The type of research is observational analytic with cross-sectional research design. The location of study was in five plantation area in Jember. Gunung Pasang (Panti sub–district), Kaliputih (Ledokombo Sub–district), Widodaren (Bangsalsari and Tanggul Sub–district), Sumberwadung and Garahan Kidul (Silo Sub–district). Those plantation areas are planted with coffee, cocoa and rubber.

The sample used in this study was 50, with details of 25 from the group of workers infected with hookworms and 25 from the group of healthy workers.

The entire sample was calculated for the number of eosinophils with peripheral blood smears. In contrast, blood plasma was taken to measure IL-4 and IgE levels measured with ELISA using Human IL-4(Interleukin 4) ELISA Kit and Human IgE(Immunoglobulin E) ELISA Kit Elabscience® with 2 repetitions. The first step is preparing a blood sample, in this examination we use plasma from the patient's blood that has been added to EDTA. Sample were centrifuged for 15 min at 1000 g × at 2~8 °C. Then the supernatant is taken for measurement using the ELISA Kit. IL-4 and IgE levels were measured using an ELISA Reader at a wavelength of 450 nm.

The data were then univariately analyzed and used the Mann Whitney test to determine the difference in IL-4 and IgE levels between infected subjects and healthy subjects.

This research was conducted at the Biochemistry Laboratory of the Faculty of Medicine, University of Jember.

Ethical approval

This research has been approved by the ethics commission of the Faculty of Medicine, University of Jember with number 1309 / H.25.1.11 / KE / 2019

Results

Based on the results of eosinophil calculations, an average of 5.43% was obtained in workers infected with hookworms, while in healthy subjects the average eosinophil was in the range of normal values (1.45%). The examination of IL-4 levels showed that in infected subjects an average of 345.8 ng/L, while in healthy subjects 24.73 ng/L. IgE levels in infected subjects averaged 471.23 ng/L, while in healthy subjects the average was 24.73 ng/L. The results of data analysis of differences in IL-4 and IgE levels between workers infected with hookworms and healthy workers showed significant differences between the two (P value = 0.000) (Table 1).

Discussion

Data analysis using Mann Whitney showed a significant difference in IL-4 levels between workers infected with Hookworm and those without ($p < 0.05$). These results are in accordance with research by Guo et al., 2015, which were the serum levels of IL-4 in patients infected with intestinal helminths (ascaris infection, hookworm infection, whipworm infection, and pinworm infection) higher than the healthy control. There was no significant difference in the serum level of IL-4 and IgE among the patients infected with the species of different helminthes. The study also showed that IL-4 and Ig E levels did not have significant differences with clinical symptoms e.g. upper gastrointestinal bleeding, abdominal discomfort or allergic dermatitis (Guo et al., 2015). Another study conducted by Amanullah et al., (2021) also showed that there was a significant difference between IL-4 levels in ascariasis-infected workers and healthy workers (Amanullah et al., 2021). Same result was also shown by Gaze et al. 2014 that hookworm infection leads to strong systemic and mucosal Th2 responses (IL-4, IL-5, IL-9 and IL-13) and regulation (IL-10 and TGF-b), with some evidence of Th1 response (IFN) -c and IL-2) (Gaze et al., 2014)

Immune response of helminth infections in humans in the acute or early phase involves induction of type 2- asscociated cytokines (IL-4, IL-5, IL-9, and IL-13) first by innate lymphocytes (ILC2) and later by effector antigen-specific polyfunctional CD4 T cells (Gazzinelli-Guimaraes & Nutman, 2018). The type 2 response is characterized by the production of cytokines such as interleukin-4 (IL-4), IL-5, IL-9, and IL-13. This response likely evolved to provide resistance by limiting the number of helminths that can live in our intestinal tract and to repair the tissue damage caused by the helminths that have colonized our tissues (Girgis et al., 2013). The polarization of the T cell response in hookworm infection is of some debate, with some studies showing a mixed Th1/Th2 response, while others report only a polarized Th2 response (McSorley & Loukas, 2010; Nair & Herbert, 2016). In acute intestinal helminthiasis, Th0 cells is proliferation and differentiation predominantly into Th2 cells. Th2 cells will express IL-4, IL-5, IL-9, and IL-13. IL-9 and IL-13 play a role in non-specific inflammatory processes, as well as elevated immunoglobulin E (IgE) (Schneider et al., 2011).

IgE levels also had a significant difference between hookworm-infected and uninfected workers ($p<0.05$). The previous study showed serum level of IgE was increased in patients of helminthiasis compared with healthy control (Guo et al., 2015).

Table 1. Results of data analysis of IL-4 and IgE levels

cytokine	sample	Average level (ng/L)	P value
IL-4	Infected worker	345,8	0,0000*
	Healthy worker	24,73	
IgE	Infected worker	471,2286	0,0000*
	Healthy worker	80,73905	

IL-4 produced by Th2 cells plays a role in helping B cells to produce IgE. B cells will also secrete more Immunoglobulin E (IgE) as part of the type 2 response. These B cells can then activate cells that express Fc receptors (FcRs), like mast cells, basophils, and eosinophils, to amplify the type 2 response by producing more IL-4 (Girgis et al., 2013). Immunoglobulin E in helminthiasis acts as an opsonin, which is to coat the target antigen (opsonization). This interaction process is known as antibody dependent cell mediated cytotoxicity / cytolytic (ADCC). Opsonization by antibodies (Ig E) causes eosinophil cells to attach to target cells or antigens. Opsonization occurs because the Fab fragment from IgE can recognize the helminth epitope, while the Fc fragment from IgE will bind to the Fc receptor on the eosinophil. As a result, eosinophils become activated and undergo degranulation which causes the release of the lysosomal protease enzyme. It is the enzyme that destroys the target cells and induces an inflammatory response to recruit phagocytes (Rusjdi, 2015).

Apart from playing a role in the resistance response to helminth antigens, IgE also plays a role in atopy protection. This is due to the modulation of the immune response to produce high levels of IgE (polyclonal IgE) which will adhere to FcεRI mast cells so that the attachment of allergen-specific IgE to mast cells is inhibited and mast cell degranulation does not occur (Daniłowicz-Luebert et al., 2011).

The result of this study can support the diagnosis of hookworm infection. The increase of IL-4 and IgE level could be as predictor of morbidity among the plantation worker, especially for hookworm infection as well as eosinophilia (Primadana et al., 2019). However, some mechanisms of immune respons in hookworm are still unclear and this could be a challenge. A literature review describing studies of the murine nematode parasite *Nippostrongylus brasiliensis*, which has important similarities to the human hookworm *Ancylostoma duodenale* and *Necator americanus*, highlights hookworm infections in recent decades of type 2 immunity with particular emphasis on how CD4 + Th2 cells Type 2 congenital lymphoid and alternative activated macrophages coordinately control helminth-induced parasitism, suggesting that a well-established cadre of leukocytes, cytokines and effector mechanisms promote clearance of helminth infections. on the other hand, clinical observations in endemic areas in individuals infected with hookworm clearly indicate that humans rarely develop secondary immunity, so further investigations into molecular and biochemical differences between human and rat hookworm species are needed (Nair & Herbert, 2016)

Conclusion

In hookworm infections, there is an increase in eosinophils up to 2 times the normal value. There was also a significant difference between IL-4 and Ig E levels of infected and healthy workers. IL-4 and IgE can be used as biomarkers to support the diagnosis of hookworm infection. These results may form the basis for further research into the effects of IL-4 and Ig-E during hookworm infection particularly in plantation workers.

Conflict of Interest

There is no conflict of interest in this study.

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Author Contributions

BH, YA, and WSU designed the study. BH wrote the article, YA did the laboratory examination, and WSU did the sampling process. All author contribute to the writing of articles.

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