

NurseLine Journal

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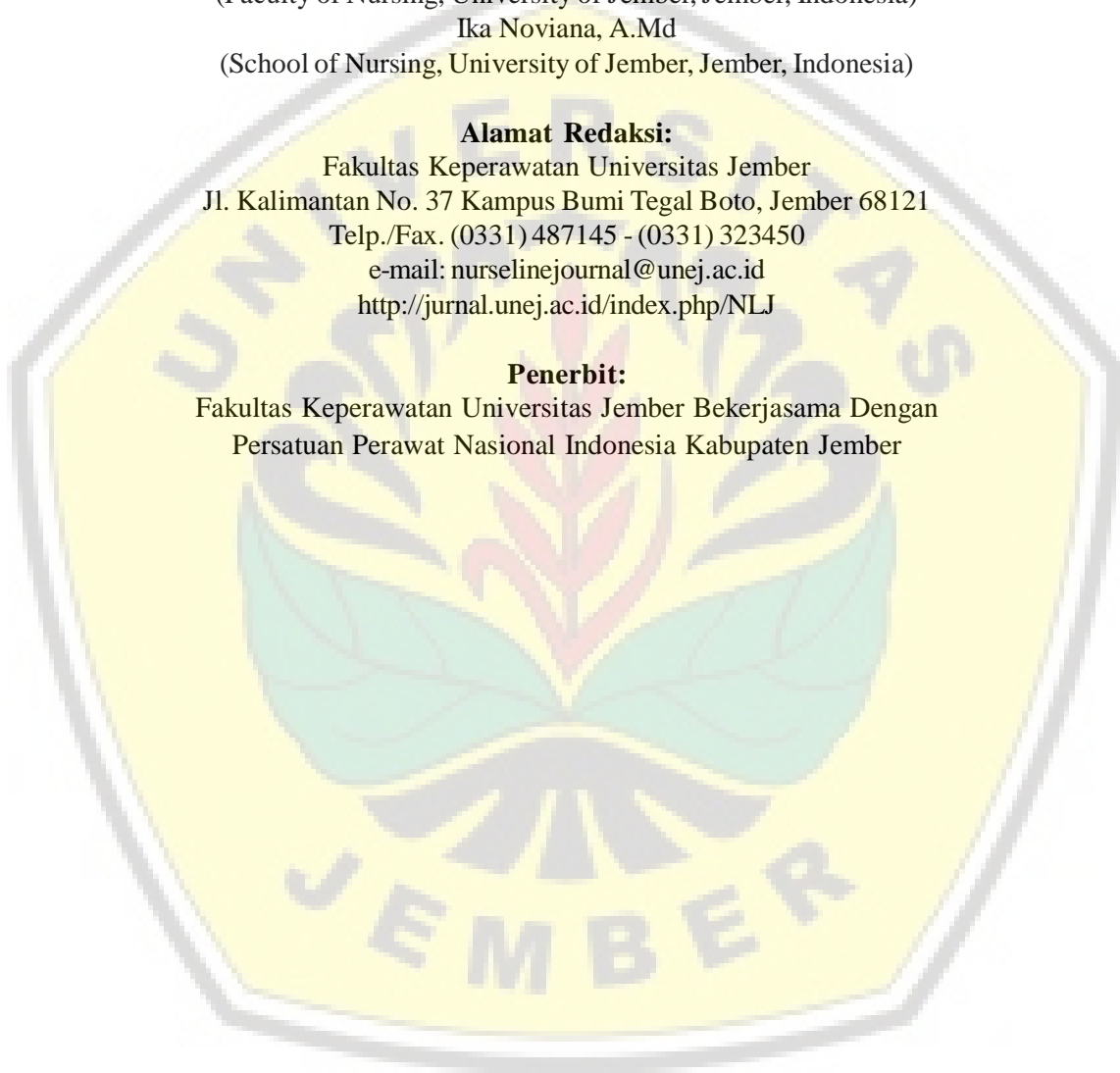
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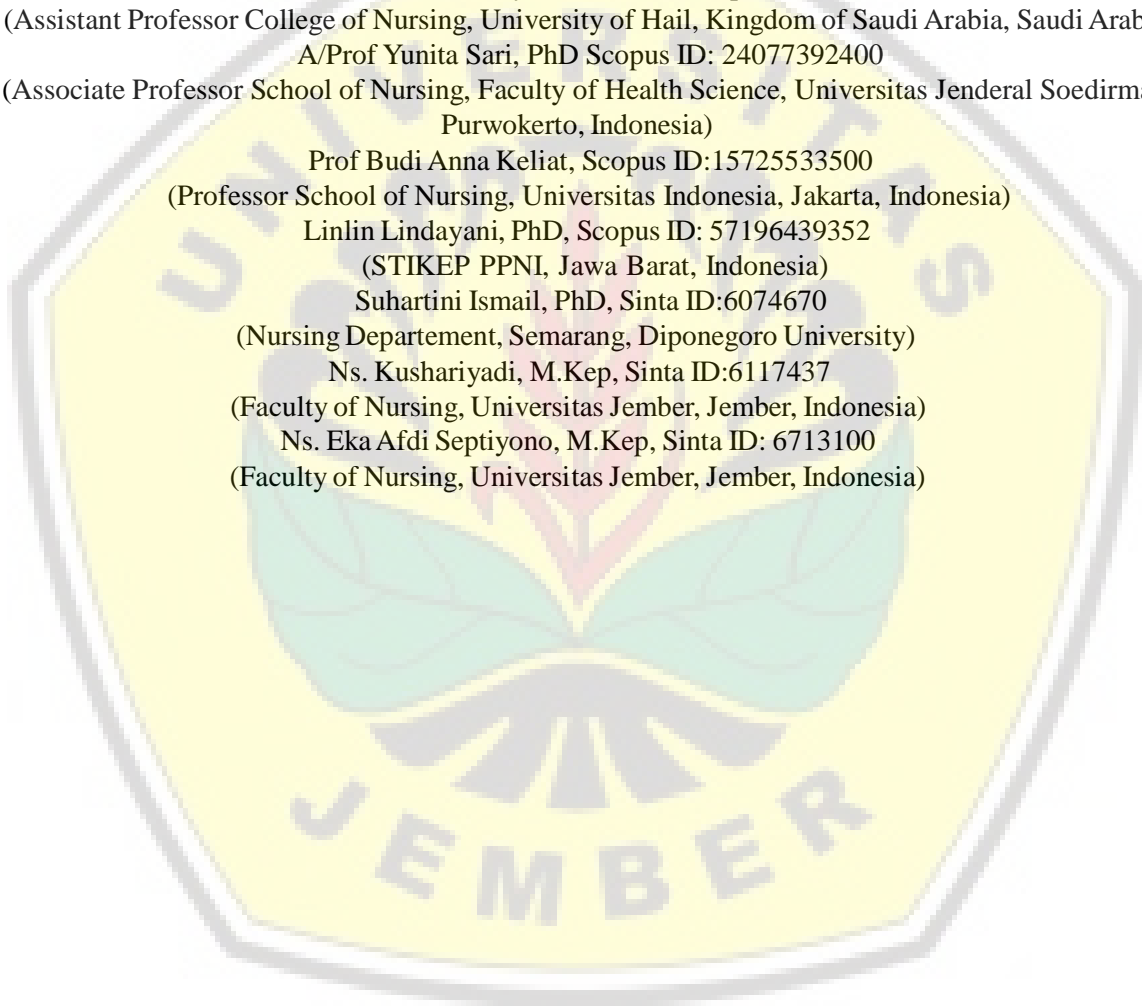
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THE DIFFERENCE OF EOSINOPHIL AMOUNT IN TUBERCULOSIS PATIENTS WITH AND WITHOUT SOIL-TRANSMITTED HELMINTHS CO-INFECTION IN PANTI DISTRICT, JEMBER

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ABSTRACT

Keywords:
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Tuberculosis is one of the world's health problems, even in Indonesia. The immune response that needed to eliminate TB can be influenced by other infections, such as helminth infection. The helminth infections induce Th2 immune responses and eosinophilia to eliminate the helminths. Meanwhile, the Th2 response and eosinophilia can also suppress the Th1 immune response, which is very important to eliminate the bacterium *M. tuberculosis* and make a negative impact on the success of TB therapy. Thus, the eosinophil profile can describe the immune response in TB patients with helminth co-infection. This study aims to determine the prevalence of helminthiasis in TB patients and determine the differences in eosinophil amount in tuberculosis patients with and without helminth co-infection. This study was an observational analytic study with a cross-sectional design conducted at Panti local health center in August 2019-January 2020 using fecal and blood samples from 24 research subjects. Helminth co-infection status was obtained from fecal examination with sedimentation and flotation methods while the number of eosinophils was obtained from leukocyte counts. Data analysis used the Mann Whitney U test to determine differences in the number of eosinophils in the two groups. The results showed there was no difference in the number of eosinophils in TB patients with helminth co-infection and without helminth co-infection ($p > 0.05$). There was no typical picture of the number of eosinophils in both groups so that eosinophils cannot describe the immune response that arose in TB patients with helminth co-infections in Panti district, Jember. Further study is needed to measure IgE and interleukin (IL4 and IL10) to know the immune response in TB patients with helminth co-infection.

BACKGROUND

Tuberculosis is a contagious infectious disease caused by the bacterium *Mycobacterium tuberculosis*. Tuberculosis is a world health problem, with 10.4 million incident cases in 2016 and is still the 10th highest cause of death in the world (World Health Organization, 2018). Jember Regency ranks second in the regency with the highest tuberculosis incidence rate in East Java (East Java Provincial Health Of-

fice, 2018). The Panti District is the 9th district with the highest number of cases from 31 districts in Jember in 2015 (Hikma et al., 2016).

The immune system can influence the success of TB therapy, and a depressed immune system will inhibit the recovery process itself. One of the things that can suppress the immune system against *Mycobacterium tuberculosis* is the presence of helminth infection in TB patients, and the most are soil-transmitted helminths. The existence of agricultural

or plantation land is one of the risk factors for STH infection. Panti District has 12,920.9 Ha of agricultural land and plantations area, which is 37.51% of the total area (Central Statistics Agency of Jember Regency, 2019).

CD4+ T cells are crucial in the immune response against *M. tuberculosis*, and these cells will differentiate into a Th1 subset. The Th1 immune response is characterized by IFN- γ production, which facilitates the development and increased activation of macrophages to deal with *M. tuberculosis* in the early stages of infection (Baratawidjaja dan Rengganis, 2018; Martino et al., 2019). Helminth infections induce Th2 immune response that can modulate the immune response needed for TB protection, which is the Th1 cells (Gashaw, 2018). The immune response caused by helminth infections tends to be antagonistic to that caused by *M. tuberculosis* infection (Mkhize-Kwitshana et al., 2017). Th1 and Th2 immune responses mutually influence each other, both in vitro and in vivo. Interferon- γ will inhibit Th2 response while IL-4 inhibits Th1 response (Jourdan et al., 2018; Yuliana, 2018). The helminth-infected person showed the lower IFN- γ (Mkhize-Kwitshana et al., 2017). This condition can affect the course of the disease and the success of TB therapy because intestinal helminth infections suppress the immune response needed to fight TB.

Another study in TB patients with helminth co-infection revealed that chronic helminth co-infection reduces the proinflammatory cytokine response while stimulating an immune response mediated by regulatory T cells (Treg) or Th2 cells (Mkhize-Kwitshana et al., 2017). Regulatory T cells appear to control of T cells and prevent autoimmune responses (Gashaw, 2018). However, these Treg cells produce IL-10 and Transforming Growth Factor- β (TGF- β) that also suppress cellular response or Th1 subset (Rusjdi, 2009). Therefore, in a chronic condition, helminth infection can also affect immune cells' ability to fight TB germs.

Eosinophil plays a role in eliminating helminth that enters the body. Eosinophils bind to IgE through the mechanism of antibody-dependent cell cytotoxicity (ADCC) to eliminate intestinal worms by secreting toxic granules (Ramirez et al., 2018). Therefore, helminth infection can trigger an increase of eosinophils (eosinophilia) (Garg et al., 2017; Kovalszki dan Weller, 2018; Ramirez et al., 2018). A study in Uganda found that the condition of eosinophilia, strongly associated with helminth infections, is strongly associated with the risk of active TB due to suppression of the Th1 immune response (Elliott et

al., 2003; Babu dan Nutman, 2016). Therefore, the number of eosinophil in TB patients with helminth co-infection could represent the condition of the immune response, particularly the Th2 subset that suppresses the Th1 subset; thus, it induces active TB. This research is essential to conduct because there is no research on the prevalence of helminthiasis in TB patients in endemic areas such as in Kecamatan Panti, Jember. It is also essential to determine the difference of eosinophil amount in tuberculosis patients with and without soil-transmitted helminths co-infection as a representation of immune response.

METHODS

This study was an observational analytic study with a cross-sectional design conducted in August 2019 to January 2020. The population in this study were all TB patients who were actively doing the treatment at the Panti local health center as many as 49 people. Sampling in this study conducted by a total sampling technique. All of TB patients at the Panti local health center were involved as participants, except pregnant patients, children aged <12 years, seriously ill until they cannot respond, suffer from chronic immunosuppressive diseases, and patients who cannot collect complete samples. We collected fecal and blood samples at the TB Clinic at Panti local health center, Jember. This study has received ethical approval from the health research ethics committee at the Faculty of Medicine, the University of Jember, with the number: 1.358/h.25.1.11/KE/2020.

The research subjects before collecting fecal and blood samples, must sign an informed consent sheet. The STH infection was confirmed by a qualitative stool examination using sedimentation and flotation methods at the Parasitology Laboratory, Faculty of Medicine, University of Jember. The data of eosinophil counted by making peripheral blood smears and manually performed the differential leukocyte counts at the clinical pathology laboratory. The results obtained will be expressed in percent units.

The data were analyzed using IBM SPSS version 26.0. The Mann Whitney U test analyzed the differences in the number of eosinophils in groups with co-infections and without STH co-infections. The results of the study were said to be significant if $p < 0.05$.

RESULTS

Characteristics of Research Subjects

TB patients who were actively doing the

Table 1. Sociodemographic characteristics of research subjects

Characteristic	Frequency	Percentage (%)
Gender		
Male	12	50
Female	12	50
Age		
< 15 Years (Toddlers and Children)	0	0
15-59 Years (Productive Age)	21	87,5
≥60 Years (Elderly)	3	12,5
Level of Education		
Unschooling	2	8,33
Basic (Elementary - Junior High School or equivalent)	15	62,5
Intermediate (Senior High School or equivalent)	7	29,17
Advanced (College or equivalent)	0	0
Occupation		
Unemployed	12	50
Farmer	5	20,83
Labor	2	8,33
Entrepreneur	5	20,83
Civil Servant	0	0
Monthly Income		
< 1 Million Rupiah	14	58,33
≥ 1 Million Rupiah	10	41,67
Marital Status		
Single	10	41,67
Married	14	58,33

Table 2. Characteristics of TB history of research subjects

Category	Frequency	Percentage (%)
Diagnosis of TB		
Radiography	3	12,5
Bacteriological	21	87,5
Rapid Test	0	0
Histopathology	0	0
Treatment History		
New Case	22	91,67
Relapse Case	2	8,33
Default Case	0	0
Failure Case	0	0
MDR-TB Case	0	0
Organ affected		
Pulmonal	20	83,33
Extrapulmonal	4	16,67
Diabetes Mellitus History		
Yes	3	12,5
No	21	87,5
Smoking History		
Yes	7	29,17
No	17	70,83

Table 3. Stool examination results

Variable	Frequency	Percentage (%)
STH Co-infection Status		
a. Positive	3	12,5
b. Negative	21	87,5
Helminth Species		
a. <i>Ascaris lumbricoides</i>	2	66,67
b. <i>Hookworm</i>	0	0
c. <i>Trichuris trichiura</i>	0	0
d. <i>Ascaris lumbricoides</i> + <i>Hookworm</i>	1	33,33

Table 4. Statistical test of differences in the number of eosinophils in TB patients with and without co-infection

Research Variable	Eosinophil Number						Asym. Sig. (<i>p</i> -value)
	Decreased		Normal		Increased		
	n	%	n	%	n	%	
Positive STH co-infection	0	0	2	8,33	1	4,17	0,759
Negative STH co-infection	0	0	12	50	9	37,5	

treatment at the Panti local health center are 49 patients, but there are only 24 patients involved in the study. The other 25 patients were not involved because they were not willing to be the subject of the study, entered the exclusion criteria, and were unable to collect complete samples.

The characterized of participants consists of sociodemographic data and TB history (Table 1 and Table 2). Table 1 shows that there is no gender dominance in TB patients in Panti District. Both genders show the same percentage of 50%. Most of the patients (87.5%) were in the productive age group (15-59 years). As many as 14 people or 58.33% were married. 50% of the research subjects did not work, and 58.33% of the subjects are low-income. Most of the subjects (62.5%) have a relatively low level of education at the level of primary education (elementary-junior high school level).

Table 2 shows the majority of patients diagnosed with TB through a bacteriological examination (87.5%). As many as 91.67% of patients were new cases of TB patients with the majority of organs affected were the lungs (83.33%). Not many patients have concomitant diabetes mellitus or active smokers. There were three people or 12.5% of TB patients in Panti District that have a history of diabetes mellitus, and 7 of 24 patients or as many as 29.17% have a history of smoking.

Co-infection Status of Research Subjects

Stool examination showed that 3 out of 24 or 12.5% of research subjects were infected with soil-

transmitted helminths (Table3). The most infecting helminth species was *Ascaris lumbricoides*. Two samples infected with *Ascaris lumbricoides* and mixed infections from *Ascaris lumbricoides* and *Hookworm* species in one fecal sample of TB patients

Number of Eosinophil Difference Test

Classification of the number of eosinophils is divided into three categories, which are decreased (<1%), normal (1-4%), and increased (>4%) (Sherwood, 2014). Table 4 showed a classification between the number of TB patients with and without STH co-infection who had decreased or increased the number of eosinophils, also the difference between them.

The results of leukocyte counts in 24 samples showed no decrease in the number of eosinophils in both groups. Fourteen samples are known to show normal eosinophil counts, with two of them (8.33%) being samples of positive STH co-infection groups. The results showed an increase in the number of eosinophils in 10 blood samples, e.g., one sample from the positive group of STH co-infections and another nine from the negative group of STH co-infections.

The Mann Whitney U test result showed there was no difference in the number of eosinophils between TB patients with STH co-infection and TB patients without STH co-infection ($p=0.759$).

DISCUSSION

The results of fecal examinations with quali-

tative methods showed that three out of 24 TB patients or 12.5% of the participants had STH co-infection. This result indicates that the prevalence of helminthiasis in TB patients in Kecamatan Panti is still relatively low at <20% (Kementerian Kesehatan RI, 2012). The TB patients in Panti District may have personal hygiene and environmental sanitation that already good enough. Therefore, the prevalence of helminthiasis was low. Personal hygiene and environmental sanitation are associated with the incidence of helminthiasis (Mulasari dan Maani, 2013; Mahmudah, 2017). Occupation can also affect this number of helminthiasis. Only five of the 24 research subjects who were working in the agricultural sector thus only a small number of workers who contact with soil.

Helminth infections will induce Th2 cell immune response (Yuliana, 2018). Differentiation of Th0 cells in the direction of Th2 will produce IL-4, IL-5, IL-9, and IL-13 expression. Interleukin-4 produced by Th2 cells plays a role in helping B cells produce IgE. At the same time, IL-5 triggers eosinophilia. Eosinophils and IgE in this helminth infection play a role in the process of interaction called Antibody-Independent Cellular Cytotoxicity (ADCC) (Rusjdi, 2009). ADCC occurs because helminth is impossible to digest directly by phagocytic cells. Antibodies function to coat the target antigens (opsonization) so that the eosinophil can be attached to the target antigen. This opsonization occurs because the Fragment Constant (Fc) of IgE bound to the parasite will interact with Fc-RI on eosinophils so that eosinophils are activated (Motran et al., 2018). Activated eosinophils will degranulate and release the lysosomal protease enzyme. This enzyme can destroy the target cell and cause an inflammatory response to recruiting phagocytic cells (Rusjdi, 2009; Motran et al., 2018). The increased number of eosinophils is a character of helminth infections.

This study found that there was no difference in the number of eosinophils between TB patients with STH co-infection and without STH co-infection. The result was showed by the insignificant results ($P > 0.05$). The result meant the majority of patients in the co-infection group or without co-infection having a normal number of eosinophils or no change in the number of eosinophils. Two out of three (8.33%) of TB patients in the Panti District infected with STH have a normal eosinophil number. There is no typical picture of the number of eosinophils that differentiates between the co-infection group and without co-infection group.

The result of this study is different from the

research conducted by Abate et al. (2012) in Ethiopia, which showed a significant association between the incidence of asymptomatic STH co-infections in TB patients with elevated eosinophil levels. The difference between the result of this study and the research conducted by Abate et al. (2012) due to different immune responses of *M. tuberculosis* in the body of the TB patient. The presence of Th1 and Th2 immune responses in the body also influences the production of T cells in TB patients with STH co-infection. Th1 and Th2 immune responses mutually influence each other (Jourdan et al., 2018; Yuliana, 2018). *M. tuberculosis* will cause the production of IFN- γ by Th1 that suppressed the Th2 cells. Therefore the production of IL-5 to activate eosinophils will also be reduced.

The normal eosinophils in TB patients infected with STH can also occur due to the chronic infection of STH. Eosinophilia occurs in the early stages of infection when the larvae migrate to the lung (Gabrie et al., 2016). Chronic helminth infections cause a Th2-modified response that triggers an increase in Treg cells and a decrease in IL-5 levels (Fallon dan Mangan, 2007). Treg cells will suppress Th2 production so that the production of eosinophils also decreases to prevent excessive immune reactions. Research conducted in Surakarta showed the same result. This result could be due to the peak eosinophilia occurring when the acute phase of the helminth infection or only when the helminth invades the tissue (Bestari et al., 2017).

The normal amount of eosinophils in TB patients who are positive for STH infection can also occur if the intensity of helminth infection is mild. This matter accords with the research conducted by Jiero et al. (2015), which showed a significant correlation between eosinophil levels and STH infection intensity. Research conducted on school-age children in Honduras shows that patients with moderate-to-severe helminthiasis have higher eosinophil counts than mild intensity (Gabrie et al., 2016).

On the contrary, nine TB patients without STH co-infection experienced an increase in the number of eosinophils in this study. The increase in the number of eosinophils can be caused by an immune response to eradicate the bacteria *M. tuberculosis*, and eosinophils also play a role in the process. The eosinophils in tuberculosis have a role as antimycobacterial. They also will trigger *M. tuberculosis* lysis by cationic protein eosinophils consisting of MBP, EPO, EDN, and ECP. Macrophages contain EPO and show intense antimycobacterial activity in an in vitro study (Prakash Babu et al., 2019). A study

conducted on 44 pulmonary TB research subjects and 44 latent TB research subjects showed an increase in MBP and EDN levels in pulmonary TB patients and a decrease in eosinophil granule protein observed after being treated with Anti-Tuberculosis Drugs (Moideen et al., 2018). Eosinophils also produce defensins, which are antimicrobial peptides in response to the Mycobacterium in infected patients (Khatun et al., 2018). These researches show that the Mycobacterium can also induce eosinophilia.

CONCLUSION

The prevalence of helminthiasis in TB patients in the Panti District is still relatively low (12.5%). There was no significant difference in the number of eosinophils in TB patients with and without STH co-infection in Panti District, Jember ($p > 0.05$). There was no differences of eosinophil count in the two groups, so eosinophils cannot describe the immune response condition of TB patients with and without STH co-infection, especially in Panti District, Jember. Further study needs to be done by measuring IgE and interleukin (IL-10, IL-12 and IL-4) to know the immune response in TB patients with helminthiasis co-infection. The local health center can also contribute to preventing helminthiasis in TB patients by providing worm medicine every six months, as well as providing education about clean and healthy behavior to the community.

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