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by P I B Apsari

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Correlation of Eosinofil and Basophil count with intensity of soil transmitted helminth infection among farmers in Bali

P I B Apsari[1*], H Arwati[2] and Y P Dachlan[3]

[1]Departement of Microbiology and Parasitology Faculty of Medicine, Universitas Warmadewa, Denpasar-Bali, Indonesia
[2]Department of Parasitology, Faculty of Medicine, Airlangga University, Indonesia
[3]Department of Immunology Faculty of Medicine, Airlangga University, Indonesia

*Putuindah51@yahoo.com

Abstract: In STH infection, the most important innate immune response on resistance to worm infections are eosinophils and basophils. The purpose of this research is to examine the correlation of eosinophils and basophils with intensity of STH infection among farmers in Bali. In this cross-sectional analytic study, 162 fecal samples from farmers aged 25-80 years were examined by Kato-Kats thick smear method and determined infection intensity based on number of eggs per gram of feces (EPG). Twenty blood samples from STH infected farmer and 20 blood samples from uninfected farmers were examined by flowcytometry method to obtain eosinophils and basophils. The results showed that prevalence of *A. lumbricoides* single infection was 1.85%, 9,3% of *T. trichiura,* 0,61% of Hookworm, mixed infection between *A. lumbricoides* and *T. trichiura* were 1.2%, mixed infection of *A. lumbricoides* and Hookworm were 0.61%. There was significant difference of eosinophils and basophils between STH-infected group and uninfected group ($p > 0.05$). Significant correlation was found between eosinophil count and EPG (r_s = 0,572, p = 0,00), also between basophil count and EPG (r_s =0.50, p = 0.001). Eosinophils and basophils are significantly correlated with intensity of STH infection among farmer in Bali.

1. Introduction

Soil-Transmitted Helminth (STH) infections remain a problem in many parts of the world, especially in developing countries with poor sanitation and environmental hygiene [1]. According to WHO (2017), more than 1.5 billion people or 24% of the world's population were infected with STH [2,3]. The infections are spread throughout the tropical and subtropical regions with the largest number infected in sub-Sahara, Africa, America, China and Asia regions. The most common STH infections in humans are with *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm) and Hookworm (*Ancylostoma duodenale* and *Necator americanus*) [4]. In 2011, the prevalence of STH infection in a rural population of Bali was relatively high: 74% for *A. lumbricoides,* 35% for hookworm, and 63% for *T. trichiura* [5]. According to the previous study in Ghana (2016) farmers had three times risk increase of STH infection than non-farmers. STH infection can disturb absorption of food and micronutrient in gastrointestinal tract, resulting in low performance of activity, productivity, and poorly condition of subject. In chronic infection, STH also impaired immune response to Malaria, Tuberculosis, HIV/AIDS, and Vaccination. The morbidity of ST infection depends to an intensity of infection [6].

The intensity of STH infection is the number of eggs per gram of feces that are counted by quantitative methods such as Kato-Kats, Mc Master, FLOTAC and concentration methods [7]. The intensity of STH infection consists of mild, moderate, and severe intensity. In ascariasis, low-intensity infections range from 1 to 4,999 eggs per gram of feces, moderate intensity of 5,000 to 49,999 eggs per gram of feces, and heavy intensity of more than 50,000 eggs per gram of feces. In trichuriasis, low-intensity infections range from 1 to 999 eggs per gram of feces, moderate intensity of 1,000 to 9,999 eggs per gram of feces, and the intensity of weight is more than 10,000 eggs per gram of feces[3]. In ankylostomiasis and nekatoriasis, low-intensity infections are 1 to 1,999 eggs per gram of feces, moderate intensity of 2,000 to 3,999 eggs per gram of feces, and heavy intensity of more than 4,000 eggs per gram of feces [3,7].

STH infection induces host immune responses such as increased immunoglobulin E (IgE), mast cells, eosinophils and basophils to combat the worm infection [1,2,5]. IgE is thought to play an important role in the protective immunity against worm infections. The bond between the worm surface antigen and the specific IgE activates effector cells through binding with FcεRI receptors resulting in Antibody Dependent Cellular Cytotoxicity (ADCC) mechanism [2]. Activated effector cells including eosinophils, mast cells, and basophils are subsequently degranulated and secrete mediators. The functions include killing parasites, inducing differentiation of naive T helper cells (CD4 +) into T helper 2, and affecting the intestinal physiology that leads to worm expulsion mechanism. Th2 cells release cytokines such as interleukin 3 (IL-3), IL-4, IL-5, IL-9, IL-13, IL-22, IL-25. IL-5 stimulates the bone marrow to produce large amount of eosinophils which migrate to the site of infection, attach to the surface antigens of the worms and release toxic granules (eosinophil secondary granule proteins/ ESPGs) that directly kill the worms [2,5]. In addition, it also has the activity of cell remodeling and cell debris cleansing, resulting in wound healing after the invasion of the worm. IL-3 stimulates the bone marrow to increase basophil cells hemopoisis and basophil cells proliferation in peripheral circulation. Basophils play a role in the secretion of T helper 2 cytokines such as IL-4 and IL-13 that affect the intestinal physiology that causes the worms to be under a stress condition [1,2]. Thus, the worm are expelled out of the body. However, the high number of eosinophils and basophils in STH infection can not completely eliminate the infection. It is shown that the prevalence of STH infection is still high in some regions [5]. So, it is important to investigate the number of eosinophils and basophils in correlation either eliminate or decrease the intensity of STH infection in farmers who are high risk persons for STH infection. The purpose of this research was to analyze the correlation of eosinophil and basophil with the intensity of STH infection among farmers in Klungkung District.

2. Materials and methods

This study was conducted in December 2017 until January 2018. The design of the study is a descriptive analytical research and implemented with the Cross-sectional design. Data were collected from adult farmers in Gelgel village, Klungkung regency, Bali, Indonesia.

2.1. Population and sample

This research was conducted at Gelgel village, Klungkung regency, Bali, Indonesia. Fecal analyzed was conducted in Parasitology laboratory, Udayana University. Eosinophil and basophil count were measured in Clinical Pathology laboratory, Sanglah Central Hospital. Total 250 farmers in Gelgel village were selected by simple random sampling technique, and only 210 samples were eligible according to the inclusion criteria. Forty samples were excluded due to health problems, such as Diabetes mellitus, Tuberculosis, Stroke. Total 162 samples were provided with a complete questionnaire and fecal container.

2.2. Ethical consideration

Ethical department Faculty of Medicine Airlangga University approved this study and released ethical certificate no.294/EC/KEPK/FKUA/2017. The head of Gelgel village also approved this study. They approved this study because there are so many occupants become a farmer, and they also wanted to improve farmer healthy.

2.3. Data collection

Stool, blood samples and questionnaires were collected from farmers by informed consent. Blood sample collection was performed after the diagnosis of STH infection in their stool either positive or negative definitely. The intensity of infection was determined by Kato-Katz thick smear method resulted in the number of eggs per gram of feces (EPG). Blood samples were used for measuring the total of IgE level by enzyme linked fluorescent assay (ELFA) (Vidas, Biomerieux, USA) and for counting the number of eosinophils and basophils by flowcytometry method (Cell Dyn Rubi, Abbott, USA). The fecal container was given to the subject, and was collected the next morning. The fecal container then filled by formalin of 10% and saved in sample the box. The fecal specimen was examined by Kato-Katz thick smear use malachite green technique to define egg of each species and to count egg per gram (EPG) feces based on WHO counting method.[7] Morphology of egg per species was identified by microscopic analysis (*merk Olympus*) with lens objective 10x. The number of positive egg was multiplicated by 24.

2.4. Data analysis

All data were tested for normality with the Kolmogorov-Smirnov ($p=0.05$) and. The association of total eosinophils and basophils with the intensity of STH infection was analyzed by Pearson or Spearman correlation test and the differentiation of them was tested by independent t-test [8].

3. Results

Total of 162 stool samples from farmers were observed by Kato-Katz method, 22 farmers were positive of STH infection and 140 farmers was negative of STH infection. Twenty blood samples were obtained from farmer infected with STH, and 20 blood samples from farmer without STH infection. The result showed that single infection of *A. lumbricoides* was 1.85% (3/162), 9.26% (15/162) of *T. trichiura* single infection, and 0.61% (1/162) of Hookworm single infection. The mixed infection was detected that were 1.23% (2/162) of *A. lumbricoides* with *T. trichiura*, and 0.61% (1/162) of *A. lumbricoides* with Hookworm.

3.1. Correlation of infection intensity with eosinophils and basophils count

The number of eosinophils and basophils were significantly different between STH-infected group and uninfected group ($p<0.05$, *independent t-test*). The number of eosinophils was significantly correlated with EPG ($p = 0.00$, *Spearman test*), the number of basophil was significantly correlated with EPG ($p = 0.001$, *Spearman test*)

4. Discussion

STH infection remains a major health problem in many poor and developing countries. Consistent with the findings of previous studies, this study shows that prevalence of STH infection among farmer is 13,5% same with the previous study in Kumasi, Ghana [9]. The was because subject of this research were similar, vegetable farmer. Most of farmer consume own vegetable product that increases the risk of STH ova exposure (OR=1,25, CI 95%). The prevalence of STH infection among adult also have been reported in Nyanza, Kenya, overall 15.7% adult positive for STH infection. The possibility for adult as carrier of STH was evaluated in Akonolinga, Cameroon, infected adult might constitute a potential parasite reservoir and a source of dissemination and persistence of STH infection [10].

The prevalence of *A. lumbricoides* infection in this study was 1,85%, this result is in accordance with the study conducted in Pakistan, the prevalence of *A. lumbricoides* infection was 1.9%. They also obtained *Hookworm* prevalence ranges from 0.6% in regular farmer similar to this study (0.6%) [11]. The prevalence of *T. trichiura* infection in this study was 9.26%, in a study that conducted in Hanoi, Vietnam the *T. trichiura* prevalence was 40%, higher than this study. This is due to the geographical differences in the study area, the ability of each species of worm to live and infect and the susceptibility of each person to get infected with different worms in each region [12]. This study obtained EPG ranging between 48-160, this result is in accordance with research conducted by Amoah et al (2016) with the mean number of eggs per gram of feces in the rainy season 4-223 EPG and in dry season 3 -124 EPG. The similarity of the results obtained because the sample used has similarities with this study. The farmers of rice, vegetables and maize in Gelgel Village was similar with vegetable farmer's subject in Kumasi, Ghana. Because of same pattern every day exposed to the soil, the risk of STH infection will be higher (OR = 3.99, 95% CI: 1, 15-13,86) among farmer than non-farmer subject. Intensity of STH infection was entirely in the mild category [9].

4.1. Correlation between eosinophils and EPG

Significant correlation between eosinophils count with EPG was present in all species of *A. lumbricoides, T. trichiura*, Hookworm or mixed infections. The results of this study were in accordance with previous studies conducted in Medan and Honduras [13,14]. This was because if EPGs would be high, so eosinophils that needed to kill the parasites were high too. Eosinophils acted as effector cells drawn by specific IgE crosslinks bound to antigens on the surface of the worm's body through FcεR1. The antibody dependent cellular toxicity (ADCC) mechanism will invite eosinophils to release its granule content such as eosinophil protein granule (ESPG), matrix metalloproteinase (MMP), ROI, and protease whose function destroyed the worms into pieces and removed from the host body [15].

4.2. Correlation between basophils and EPG

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Significant correlation between basophil count and EPG could be an effect due increasing Th2 cytokine such as IL-4 and IL-13 as a protective host immune response to worm infections. Intestine lumen that induced by IL-4 and IL-13 would undergo physiological changes like increased permeability, increased smooth muscle contraction so that the peristaltic of the intestine could increases, increased epithelial cell proliferation and goblet cell hyperplasia so produced many mucin substance, so that the worms will be covered by many mucus and done expulsion of intestinal worm outside. [16,17]. Despite the activity of goblet cells depending on levels of IL-4 and IL-13, it also may be triggered by IL-25 and IL-22 [17,18]. Goblet cells act as a selective barrier in worm infections. The Th2 cytokine converts the sugar residue in mucin which caused stress environment of worm and prevented the development of the worm into a normal form. The ability of Th2 cells to change the mucin composition depends on the species of the worms [19].

5. Conclusion

Grounded the above obtained results, it can be concluded that the intensity of infection was corresponding with total eosinophils and basophils. It seems that the host immune response was insufficiency to eleminate the STHs. Thus, STH infections need to be treated by antihelminthic drugs. Therefore, the correlation between specific IgE level and the intensity of STH infections and also the proteins which are released by STHs should be investigated further, in order to clarify the defense mechanism of STH against to host immunity.

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