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Foreword: Assalamualaikum warrahmatullah wabarakatuh Greetings for all of you Excellency Prof. Dr. Hj. Sri Iswati, S.E, M.Si. Excellency Dr. Soekarwo S.H, M.H Excellency Dr. Ir. H. Sambari Halim Radianto, S.T, M.Si The Keynote speakers, invited speakers, presenters and participants of the conference Ladies and gentlemen Alhamdulillahirabbil 'alamin, we are here to The Second International Conference Postgraduate School with the theme Innovation of Technology and Bureaucracy

Towards Good Governance to improve the Nation's Competitiveness. Universitas Airlangga is delighted to welcome you all in this two day conference. Ladies and gentlemen A university role is one of many pillars to build a nation that has great civilization, government system, human resources, natural resources, and other potentials It is our roles as academics and as a part of the university in maximizing the potentials to generally develop our nation or to specifically increase our nation's **(More)**

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Correlation between Parasite Density with Plasma Levels of TNF-α and IL-10 in Malaria Mix Infection in East Sumba District, East Nusa Tenggara Province

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Keywords: Mix Malaria, Parasite Density, TNF-α, IL-10

Abstract: East Nusa Tenggara Province is a malaria endemic area located in Eastern part of Indonesia with Annual Parasite Incidence of 7.04% including falciparum malaria, vivax malaria and mix malaria. The information on parasite density and plasma levels of TNF- α and IL-10 in mix malaria infection is rarely found. This research is conducted to collect the information mentioned above. Methods: Diagnosis of malaria infection in subjects was done by Rapid Diagnostic Test (RDT) as well as microscopy examination. Parasite density was calculated based on number of parasite per 200 leukocytes on the Giemsa-stained thick blood films. Levels of TNF-α and IL-10 in plasma were measured using Enzyme-Linked Immunosorbent Assay (ELISA). Results: Five patiensts were diagnosed as mix malaria infection, with average of parasite density was 4341 paracite/µL. Average level of TNF-α and IL-10 was 207.31 pg/mL and = 15.91 pg/mL, respectively. Ratio of TNF-α : IL-10 was 13 : 1. This research concludes, increased levels of TNF-α will decrease the parasite density based on the time of infection, while the increase in parasite density is directly proportional to elevate levels of IL-10 (p= 0,032).

1 INTRODUCTION

Malaria is a long-standing disease that still threatens the lives of children, pregnant women and many others in East Nusa Tenggara (ENT). Indonesian malaria reports show that the population of ENT, which accounts for 2% of Indonesia's population, contributes to 25% of the total incidence of malaria in Indonesia. On the island of Sumba itself the Annual Parasite Incidence is high (Pusdatin, 2016).

Indonesia is located in a tropical which is the endemic area of *P. falciparum* and *P. vivax* and high transmission rate of malaria through mosquito bites makes it possible for repeating inoculation by both *Plasmodium* and causing mixed infection (Imwong, 2011).

Clinical conditions of people infected with mixed malaria differ from those infected with single malaria, this is due to the interaction between *Plasmodium* in human hosts. The *P. falciparum* parasite has a faster life cycle than *P. vivax*. The temperature of fever with mixed infection is higher than single infection (McKenzie, 2006).

The aim of this research is to determine the relationship between parasite density with plasma levels of TNF-α and IL-10 in patients with P. vivax and P. falciparum mixed infections in East Sumba Distric, East Nusa Tenggara.

2 IMMUNE RESPONSE DURING MALARIA INFECTION

2.1 Innate Immune Response

Innate immune system is the first step in the immune response to pathogens. The system includes a variety of nonspecific responses such as recruitment of immune cells to the site of infection, complement cascade activation, destruction of foreign objects by specific leukocytes and activation of the adaptive immune system through antigen presentation (Clark, 2010). The main function of innate immunity is to limit the parasitic density of the initial infection and to modulate the specific immune response necessary to eliminate parasites (Stevenson, 2004).

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Non-specific immune responses are represented by multiple cells and intercellular components. One of the innate immune systems that will respond during a malaria infection is the mononuclear phagocyte system (MPS), which includes monocytes, macrophages, and dendritic cells (Mac-Daniel, 2015).

2.2 Adaptive Immune Response

2.2.1 Cellular

In the cellular immune response, T lymphocytes are a major component of the immune system that serves to recognize and destroy antigens through cytotoxic activity and cytotoxic activation and activation of various cell types and cytokine production (Abbas, 2012).

2.2.2 Humoral

The main characteristic of the *Plasmodium* parasite that infects the erythrocytes is increasing the production of Tumor Necrosis Factor alpha (TNF-α) cytokines from macrophages, malaria pigments and glycolipids such as Glycosil Phosphatidyl Inositol (GPI). TNF is a major cytokine in acute inflammatory responses. Severe infections can trigger the production of TNF in large quantities that lead to systemic reactions. TNF-α is produced by neutrophils, activated lymphocytes, macrophages, Natural Killer (NK) cells and some non-lymphoid cells such as astrocytes, endothelial cells and smooth muscle cells (Dietrick, 2008).

TNF-α plays a role in regulating Interleukin 12 (IL-12) production by macrophages and shows that TNF-α is important as a co-factor for IL-12 in increasing Interferon (IFN) production by NK cells. TNF-α concentrations in plasma are associated with changes in fever and parasite clearance (Malaguarnera, 2002).

IL-10 that produced by monocytes is found in plasma of patients with acute malaria. Th2 cells and B cells, inhibiting cytokine production in Th1 and CD8+ cells. IL-10 increases cell proliferation and immunoglobulin production necessary for the development and maturation of anti-malarial antibodies. IL-10 also serves as a down regulator in macrophages, reduces antigen presentation, plays an important role in neutralizing the pathology of macrophages in cerebral malaria by inhibiting IFN-γ and TNF-α secretions (Malaguarnera, 2002).

3 METHOD

3.1 Location

This research was done in East Sumba district of the Province of ENT. This province is located in Eastern part of Indonesia that contain 7 islands. East Sumba distric also contains several small islands. Easter Sumba district bordered by the Sumba strait on the north, Hindia ocean to the south, Sabu sea to the east and Central Sumba district to the west. This district that located in tropical region has rainy during January-April and the rest was dry season, causing this region classified as dry area (BPS Sumba Timur, 2016).

3.2 Blood Samples Collection

Blood sample colletcion was done by active and passive malaria case detections. Active case was performed by visitsing malaria suspected patient in high transmission areas. Passive case detection was carried out by collecting the blood samples of patients who visiting Public Health Centers (PHC) and Lindimara Christian Hospital. Prior to blood colletion the characteristics of patients were recorded including name, gender, age and address. All individuals aged from four to seventy years old of both sexes included in the study. Three millilitres of blood were collected by vena punctured after the patient signed the informed consent to prepare thick and thin blood film and plasma collection. Inclusion critera were people who diagnosed positive mixed malaria infection containing *P. falciparum* and *P. vivax* by Rapid Diagnostic Test (RDT) and microscopy examination.

3.3 Microscopy Examination and Parasite Density Count

Microcopy examination was done on Giemsa-stained thick and thin blood film using light microscop under 1000x magnification with oil immersion to detect and identify the species of malaria parasites. Detection by RDT was conducted by the staff of PHC. Parasite density were counted per 500 leucocyte based on the following formula:

$$Parasite\ Density = \frac{\sum Parasite\ x\ 8000}{500} \quad (1)$$

3.4 Enzyme-Linked Immunosorbent Assay (ELISA)

Plasma levels of TNF-α and IL-10 were measured by using ELISA according to the manufacturer's protocol (Elabscience, USA), with all samples running in a single assay. The ELISA was performed andnd analysed by a single operator, and standard curves were derived from cytokine standards.

3.5 Data Analysis

A total of 110 individuals enrolled in this study, 64 were diagnosed negative malaria, 22 diagnosed as positive P. falciparum, 19 diagnosed as positive P. vivax and 5 diagnosed as positive mixed malaria.

4 RESULT AND DISCUSSION

4.1 Thick Blood Smear Examination

Subject diagnosed as positive mixed malaria have 2 types of *Plasmodium* that is *P. falciparum* and *P. vivax* can be seen under microscope. The result of thick blood film examination as follow:

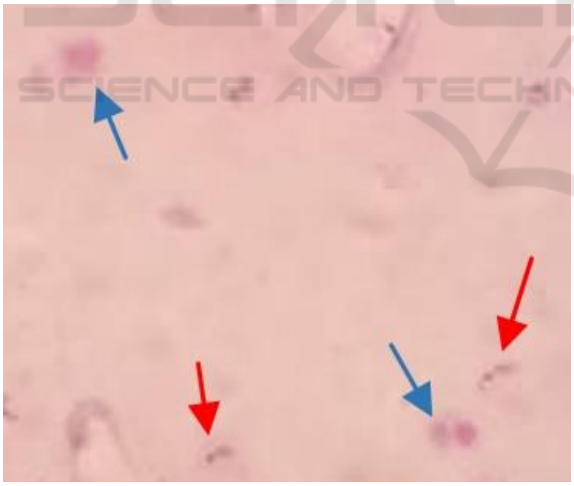

Figure 1. Microfotography of mixed malaria thick blood smear

Seen in Figure 1. the difference between the two species of the ring and the trofoocytic nucleus P. vivax is thicker and larger (blue arrow) than the ring and nucleus of the thin and small P. falciparum trophosoit (red arrow)

4.2 Parasite Density

Five samples were examied with ELISA and found the Parasite Density as follow:

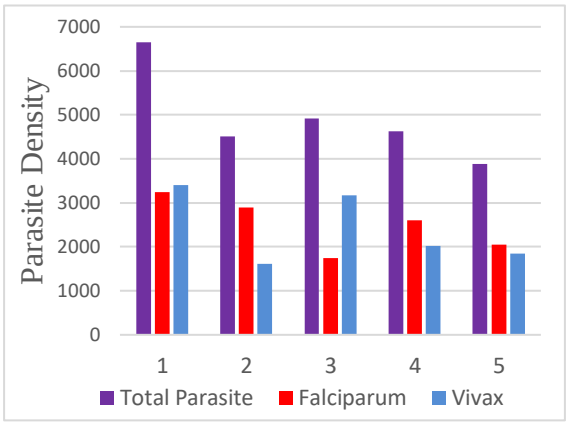

Figure 2. Parasite density of subject with mixed malaria

The highest parasitic density was 6,656 parasites/μL while the lowest parasitic density was 3,888 parasites/μL. From Figure 2. the mean ratio between P. falciparum and P. vivax is 1.04: 1 indicating no dominant *Plasmodium* in mixed infections found.

4.3 Plasma Cytokine Level

Five samples were examined with ELISA and found the level of of TNF-α and IL-10 as follow:

Table 1. Plasma cytokine level of mixed malaria

No.	TNF- α	IL-10
1.	0	10,523
2.	94,861	35,559
3.	0	0
4.	101,085	0,807
5.	425,983	11,369

Subjects 1 and 3 have very low levels of TNF-α and can not be detected with ELISA. Subjects with low TNF-α levels were subjects 2 and 4 of 94.861 pg/mL and 101.085 pg/mL respectively, whereas highest TNF-α levels were in sample 5 of 425.983 pg/mL. Subjects 1 and 5 had relatively low IL-10 levels of 10.523 pg/mL and 11.369 pg/mL while the highest IL-10 levels were in sample 2 of 35.559 pg/mL. Subject 4 had a very low IL-10 level of 0.807 pg/mL and subject 3 was very low and undetectable at ELISA. From Figure 2. can be determined the ratio of TNF-α and IL-10 for each subject. Subject 1 has ratio TNF-α : IL-10 of 0, subjet 2 ratio 2,67:1, subject

3 ratio 0, subject ratio 125,15:1 and subject 5 ratio 37,47:1.

4.4 Statistical Analysis

Statistical analysis were conducted to data from 5 subjects. Kolgorov-Smirnov test were used to describe the distribution of samples. The results of these tests show the distribution of the sample is normal with $p > 0,05$.

With normal distribution sample, next analysis is using Pearson corellation test to determine the correlation between parasite density, TNF-α, IL-10 and the ratio of both cytokines. Correlation is significant at the $p < 0,05$. The result shows that no significant correlation between parasite density and TNF-α with $p= 0,213$ also no significant correlation between parasite density and cytokine ratio with $p= 0,130$. But there is a significant correlation between parasite density and IL-10 with $p= 0,032$.

4.5 Discussion

Increased levels of IL-10 correlate with increased parasitemia. Exemplified by the highest parasite density in subjects No. 1 compared to 4 other subjects. The increase in IL-10 accompanied by very low levels of TNF-α shows IL-10 anti-inflammatory cytokines suppress the performance of TNF-α as a pro-inflammatory cytokine in eliminating parasites (Othoro, 1999). Subjects No. 2 showed an imbalance of pro and anti-inflammatory cytokines responses because with parasite densities that were still 4,512 parasites / µL the levels of IL-10 had increased to reduce the TNF-α: IL-10 ratio of 2.67: 1, if IL-10 levels were continues to increase whilst TNF-α level were decreased, the correlation was parasite density in subject No.2 would not decrease. Plasma level of IL-10 subjects No.4 need to be re-examined to see the direction of change, if IL-10 remains in a low state then TNF-α levels will be uncontrolled and at risk of causing malaria complications. If IL-10 levels increase optimally, there will be a regulatory balance between pro-inflammatory and anti-inflammatory cytokines. Subjects No.3 has no detectable plasma cytokine level, this correlates with the parasite density that still considerably high because no cytokines response from the subject. Subject No.5 has a clinical manifestation of high body temperature, this indicates that level of TNF-α were increased thus inhibiting the growth of parasite. This correlation can be seen by the Parasite Density of Subject No.5 was the lowest among other subjects.

5 CONCLUSIONS

Increased levels of TNF-α will decrease the parasite density based on the time of infection but an excessive increase will lead to complications of malaria. While the increase in parasite density is directly proportional to elevate levels of IL-10 is evidenced by statistically significant correlation between parasite density and IL-10 ($p = 0,032$).

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