Correlation of Parasite Density with Plasma Level of TNF-a and IL-10

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Submission date: 06-Mar-2023 11:56AM (UTC+0800)
Submission ID: 2029827029
File name: ion_of_Parasite_Density_with_Plasma_Level_of_TNF-a_and_IL-10.pdf (282.85K)
Word count: 2072
Character count: 11378

Correlation of Parasite Density with Plasma Level of TNF-α and IL-10 in Patients Infected by Plasmodium *Vivax* in East Sumba District, East Nusa Tenggara Province

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Keywords: IL-10, Parasite Density, Plasmodium *vivax*, TNF-α.

Abstract: Introduction. Annual parasite incidence (API) in East Nusa Tenggara Province (NTT) 2015 per 1000 population is 7.04%. However, API in each Public Health Center (Puskesmas) Sumba Island remains high. High levels of pro-inflammatory cytokines in malaria infection, such as TNF-α is associated with severe pathology, whereas, anti-inflammatory cytokines such as IL-10 is associated with acute malaria. The objective of the study was to analyze correlation between parasite densities and plasma level of both cytokines in P. *vivax*-infected patients in East Sumba Regency East Nusa Tenggara Province. Methods. Parasite densities were calculated per 500 leucocytes on Giemsa-stained thick blood smears. The levels of TNF-α and IL-10 were measured by Enzyme-Linked Immunosorbent Assay (ELISA) method. Statistical analyses were done by Spearman test. Results. Correlation was observed significantly in parasite density and TNF-α $p = 0.032$ and parasite density and IL-10 $p=0.000$. This result indicated that the stage of immunity in patients was not affected by the parasite density but clinical symptoms may have a greater role in increasing and decreasing the plasma level of cytokines. Conclusion. There was correlation between parasite densities and plasma level of TNF-α and IL-10 in P. *vivax* infected patients is the studied areas.

1 INTRODUCTION

Malaria incidence was still high in the eastern parts of Indonesia including Papua Province, West Papua, East Nusa Tenggara (NTT), Central Sulawesi and Maluku (Kemenkes, 2013). During 2015 the Annual parasite incidence (API) in East Nusa Tenggara Province (NTT) per 1000 population was 7.04%, the number of cases of positive malaria as high as 36,039 from 5,120,061 inhabitants. The API in each Public Health Center (Puskesmas) in Sumba Island remains high (Pusdatin, 2016).

Malaria has been known since 3,000 years ago and is caused by protozoa of the genus Plasmodium and transmitted by female Anopheles mosquitoes (Gunawan, 2000). There are 5 species of parasite causing malaria in humans, namely Plasmodium *falciparum*, Plasmodium *vivax*, Plasmodium *malariae*, Plasmodium *ovale* and Plasmodium *knowlesi* (White et al., 2014).

Plasmodium *vivax* has a longer incubation time (12 days to several months), has a erythrocyte cycle 42-48 hours and produces fewer merozoites per schizon. It is generally known that P.*vivax* requires a duffy antigen as a receptor needed to invade host erythrocytes. In humans who do not have this antigen, they will become resistant to the infection (Andrade et al., 2010).

An immune response to malaria leads to parasite elimination or persistent responses are mediated by cytokines that cause immunopathology. In malarial infection high levels of pro-inflammatory cytokines, such as Tumor Necrosis Factor (TNF), Interferon Gamma (IFN-γ) and Interleukin-6 (IL-6) are associated with severe pathology whereas cytokines, anti-inflammatory agents such as Transforming Growth Factor Beta (TGF-ß) and IL-10, are associated with acute malaria. IL-10 cytokines have an important role as immuno-regulators from infections caused by Plasmodium, by neutralizing theeffects of cytokines produced by Th1 and CD8+ cells, which are responsible for immunopathology associated with excess cytokine production (Medina et al., 2011).

Nela, F., Arwati, H. and Dachlan, Y.
Correlation of Parasite Density with Plasma Level of TNF-Îš and IL-10 in Patients Infected by Plasmodium Vivax in East Sumba District, East Nusa Tenggara Province.
DOI: 10.5220/0007542203250328
In *Proceedings of the 2nd International Conference Postgraduate School (ICPS 2018)*, pages 325-328
ISBN: 978-989-758-348-3

Activated macrophages release pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 (Tsokonas et al., 2002). The release of TNF-α apart from activated macrophages can also be directly induced by the malaria parasite and its dissolved antigen such as malaria pigment (haemozoin) and Glycosylphosphatidylinositol (GPI). TNF-α indirectly inhibits parasites by increasing phagocyte activity of monocytes (Wipasa et al., 2002; Korbel et al., 2004).

CD4+ T cells are classified into 2 major subsets according to the cytokine production pattern. Th1 produces IL-12, IFN-γ, and TNF-α. While Th2 produces IL-4, IL-5, IL-6, IL-10. In general, Th1 cells are responsible for cell-mediated immunity (CMI). The cytokine activates macrophages and other cells to produce mediators releasing inflammatory cytokines. Th2 cells regulate humoral immune by helping B cells to produce antibodies. Th2 cells promote the production of immunoglobulins. Both Th1 and Th2 cells are involved in protective immunity against malaria in the pre-eritrocytic stages, and the balance of cytokine production by both Th is the determining factor of the disease (Wipasa et al., 2002).

2 MATERIAL AND METHODS

2.1 Subject of Research

The blood samples were collected from East Sumba residents by active case detection in the villages with high API value. Passive case detection was done by collecting blood samples from P.*vivax*-infected patients who came to Puskesmas seeking medication. Blood samples were taken from those who meet inclusion criteria which is P.*vivax* positive by rapid diagnostic test (RDT) and microscopic examination followed by the signed informed consent.

2.2 Screening

A screening test is used to determine the inclusion criteria described above using 2 methods: RDT and microscopic tests. All samples were examined using a microscopic. RDT is used when sampling is active in the village because there is no microscope to support microscopic examination. Microscopic examination is still performed after the RDT results show a positive P.*vivax*.

2.3 Enzyme-Linked Immunosorbent Assay (ELISA)

Blood collection from vein cubitis patients with P. *vivax* malaria three milliliters (ml), inserted blood into the heparin tube, centrifuge for 15 minutes at a speed of 3000 rpm, the plasma is taken and transferred into ependorf tube using micropipette.

The measurements of TNF-α and IL10 levels use the Enzyme-Linked Immunosorbent Assay (ELISA) in accordance with manufacturer protocols, with all samples running in a single assay. The ELISA was performed and analyzed by a single operator, and standard curves were derived from cytokine standards.

3 RESULT AND DISCUSSION

3.1 Parasite Density

P.*vivax* positive samples that have been collected are smeared in thick drops and examined using a 1000x magnification microscope. The formula for calculating parasite density is as follows:

$$\text{Parasite density} = \frac{\sum \text{Parasite} \times 8000}{500}$$

The following is a microphotography picture of thick blood smear P.*vivax*:

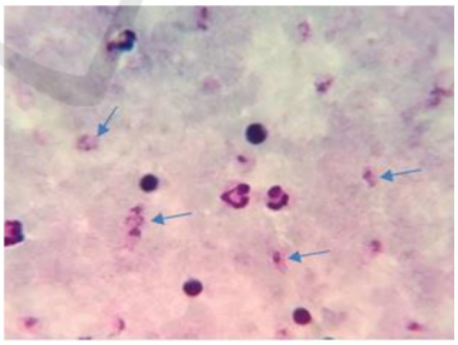

Figure 1: Microphotography P.*vivax* thick blood smear

Parasite density of samples diagnosed positive P.*vivax* as follows:

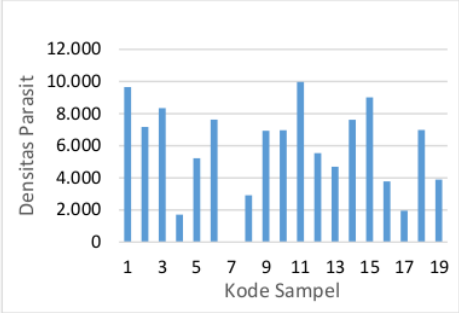

Figure 2: Parasite density P.*vivax*

3.2 Category of TNF- α and IL-10

Measurement of TNF-α and IL10 levels using ELISA can be categorized according to the following:

Table 1: Category of TNF-α levels

TNF-α (pg/ml)	Category	Frequency	%
0 – 100	Low	9	47,37
101 – 500	Intermediate	7	36,84
> 500	High	3	15,79

TNF-α level was categorized as 3 types that is low, intermediate, high. The percentage of TNF-α low is 0-100 is 47.37%, intermediate 101-500 is 36.84% and high> 500 is 15.79%.

Table 2: Category of IL-10 levels

IL-10 (pg/ml)	Category	Frequency	%
0 – 10	Low	11	57,89
11 – 50	Intermediate	7	36,84
> 50	High	1	5,26

TNF-α level was categorized as 3 types that is low, intermediate, high. Low IL-10 percentage is 0-10 is 57,89%, intermediate 11-50 is 36,84% and high> 50 is 5,26%.

3.3 Category of TNF- α and IL-10

Kolgorov-Smirnov Test was used to find out the normality of data. When the data is evenly distributed, then Pearson test was used to analyze the correlation between parasite density with TNF-α and IL-10. If the data is distributed unevenly, Spearman test was used. The correlation is significant if $p < 0.05$ is obtained. The results showed that significant correlation was observed significantly parasite density and TNF-α $p = 0.032$ and parasite density and IL-10 $p=0.000$.

3.4 Discussion

Infections caused by P.*vivax* have long been regarded as benign, especially when compared with infections caused by P.falciparum, but *vivax* malaria causes more severe disease than P.falciparum infection (Borges et al., 2013).

TNF-α is a pro-inflammatory cytokine that is the cause of fever (Hietbink et al., 2006). At high levels TNF-α can cause severe tissue damage (Couper et al., 2008). At the optimum level TNF-α can kill parasites directly, provide protection and lead to malaria recovery. Low levels of TNF-α can inhibit the growth of parasites in the stadium in the blood by activating the cellular immune system (Raza et al., 2013).

IL-10 is the main anti-inflammatory cytokine in the natural immune response and adaptive inflammatory response through the process of inactivation of macrophages and T cells (Dodoo et al., 2002). High level of IL-10 will prevent the development of severe malaria anemia (Weatherall et al., 2002). The occurrence of severe anemia is associated with a decrease in the concentration of IL-10 in the circulation and increases the ratio of TNF-α and IL-10. This condition contributes to the reversible suppression of bone marrow activity that occurs in malaria patients (Malaguarnera, 2002).

4 CONCLUSIONS

There was correlation between parasite densities and plasma level of TNF-α and IL-10 in P. *vivax* infected patients is the studied areas. This result indicated that the stage of immunity in patients was not affected by the parasite density but clinical symptoms may have more role in increasing and decreasing the plasma level of cytokines.

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