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Expression of IL-10 and IL-12 Children Suffering Malaria with Supplementation of Zink and Fe

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Abstract:- Over the past decade, there has often been a shortage of Zn and Fe simultaneously. In cell culture, Zn and Fe are micronutrient substances that can increase immune responses so that the number of malaria parasite cultures is decreased or negative. The presence of Zn and Fe as micronutrients in the body is influenced by nutritional status. A child can be considered to have a good nutritional status if the micronutrient elements in the body are fulfilled. This study aims to analyze the expression of IL-10 and IL-12 children suffering malaria by providing Zinc and FE supplementation to elementary school children in malaria-endemic area. This experimental study gave treatments to the research subjects, divided into four groups, including the treatment group with @ 20 mg/day Zinc (Zn), Fe @ 20 mg/day, Zn and Fe @ 20 mg/day simultaneously and the control group (placebo). The dose given in this study was 20 mg. This study employed the Randomized Controlled Trial Pre-Post Test Design by providing a Double-Blind treatment. Further, the data were statistically analyzed using one way ANOVA analysis with 5% reliance level (1 = 0.05). The results show that the level of IL-12 is increased after the treatment of Fe and Zn supplementation; meanwhile, the level of IL-10 is decreased. The approach that can describe this situation is the presence of seasonal malaria, low transmissibility, low percentage of API and the Zn levels which are up to normal in the 9 to 12-year-old children. This causes the increased protective IL-12 level so that the IL-10 level is no longer needed to re-stabilize the level of IL-12 so that the level of IL-10 in this study is decreased.

Keywords:- Zn and Fe, IL-10, IL-12, Malaria.

I. INTRODUCTION

Over the past decade, there has often been a shortage of Zn and Fe simultaneously. In the cell culture, Zn and Fe are micronutrient substances that can increase immune responses so that the number of malaria parasite cultures is decreased or can be negative. The main function of Zn is to synthesize the nucleic acids and proteins in the body while the function of Fe is on the production of the red blood cells. These red blood cells are necessary to transfer oxygen right through the body tissues.

The role of Zn in the body to this infection is widely known. Zn is the thymic hormone component that facilitates and controls the lymphocyte maturation. Zn also has a significant role in cell division and the replication of DNA, thus helping to produce immune system cells (Wei Fu, et., al, 2013).

Iron (Fe) plays a vital role in the immune system and defense from the infection. However, how the iron substance mechanisms influence the process in the cellular levels and molecular is unclear yet. In the immune system, Fe plays a significant role. Cell-immune responses by lymphocytes-T can be disrupted because of the decreased formation of those cells, which might be caused by the decrease of DNA synthesis and ribonucleotide reductase enzyme disorders that require Fe to perform its functions. The two key cells which are involved in performing immune functions are lymphocytes and macrophages (Calder, et.,al, 2002).

The presence of Zn and Fe as micronutrients in the body is influenced by nutritional status. A child can be considered having a good nutritional status if the micronutrient elements in the body are fulfilled. Zn and Fe will help the activation of the non-specific immune system or innate immunity to help the body's defense in facing the attacks of various microorganisms since it can provide direct responses to the antigen. Therefore, it influences the number of activated lymphocytes-T, the function of phagocytosis, the number of NK cells and level of IL-10 (Zhang, et al., 2012).

The increased number of NK cell will affect the total of IFN-γ levels in the serum so that it will be able to activate the function of phagocytosis macrophages. Macrophages produce IL-12 for the first time. The effect or mechanism of IL-12 is essential because IL-12 has the protective effect, either to kill or inhibit malaria parasites, and to activate phagocytosis by macrophages or to increase the destruction of nitric oxide. IL-10 level is required as the feedback to inhibit the excessive proinflammatory responses that may inhibit the work of macrophages, IL-12, and IFN-γ in order not to continue to Th-1 (Zhang, et., al, 2012).

Children in West Africa typically have anemia (low Hb) after they were born. Anemia may be associated with motor developmental obstacles and learning skills and may affect immunity. In countries that have many cases of malaria, it is recommended that FE supplementation should not be administered in areas with malaria because it may increase the risk of malaria and death. The High doses of Fe given as medicine can cause the increase of free Fe that is circulated in the blood and may enhance the chance of parasitic growth. High doses of Fe given as a drug can lead

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to the increased number of free Fe circulating in the blood as well as may also extend the chance of parasitic growth. A study conducted by Ojukwu et al. (2010) find out that iron supplementation does not boost the risk of malaria disease. This is proven by the emergence of fever and the existence of parasites in human's blood and there is no increased risk of death in children treated with iron.

Regarding the existence of the conflicting reports, it is necessary to examine the role of Zn and Fe supplementation given to children after the infection of malaria. This study was conducted to explain the role of Zn and Fe in preventing the recurrence of re-infection of malaria found in children after the infection. The results of research of Zn and Fe role which have been published so far are still oriented to children who are suffering from malaria disease (Ojukwu, 2010). Meanwhile, a study about children who are considered healed from malaria has never been done. Therefore, it is necessary to study the role of Zn and Fe supplementation in children after the infection of malaria with IL-10 and IL-12 as the parameters of immune responses.

The aim of research: This study aims to analyze the results of Zn, Fe along with Zn and Fe simultaneously on IL-10 and IL-12 in elementary school children after malarial infection in endemic malaria areas.

II. MATERIAL AND METHOD

This experimental research provided treatment to the research subjects. The treatments were divided into four groups; the treatment group with @ 20 mg / day Zinc (Zn), Fe @ 20 mg / day, Zn and Fe @ 20 mg / day simultaneously and the control group (placebo). The dose given in this study was 20 mg.

This study employed the Randomized Controlled Trial Pre-Post Test Design by giving a Double-blind treatment. Further, it involved elementary school children who had got the infection of malaria in malaria and endemic area as the research population. After that, this study carried out a screening process based on the inclusion criteria, as follows:

- Group of elementary school children around 9-12 years old.
- After being infected to malaria falciparum and having been treated with anti-malarial medicines.
- Preparations of Plasmodium positive blood by ICT and microscopic.
- Willing to participate in this study after understanding the ways and purposes.
- Exclusion criteria that might cause someone to be excluded from the analysis include:
- Suffering malaria with complications.
- Taking other anti-malarial medicines before or during the treatment.

Subjects in this study were elementary school children who were infected to malaria in endemic malaria area in Dulukapa village, Sumalata subdistrict. Subjects that are willing to be included in the study were provided information related to the study and were asked to sign the informed consent. In addition, the research sample was taken from the selected population by using the random sampling technique. Simple random sampling was utilized because the population was homogeneous.

A. Tools and Materials

Cotton, 70% of alcohol, anticoagulant EDTA, HNO3 5%, concentrated HNO3, concentrated HCIO3, aquabidest, aquacleonized, acetylene gas, aluminum foil (Aluminum paper), Giemsa parent, PH 7.2 phosphate buffer, immersion oil, Sterile disposable syringe 5 ml, latex handshoe, blood lancet, object glass, masks, EDTA tubes, 5 ml and 2.5 ml disposable syringes, test tubes, 1 ml microtube for serum storage, tourniquet, centrifuge to separate serum, centrifuge tubes, automatic pipette, cooler box to bring samples (Blood and serum), AAS (Automic Absorbent Spectrophotometer), electric stove / electric heater / coil of Maspion 600 watt, Microplate reader that is able to measure the absorbance at 450 nm with wavelength correction arranged at 540 nm or 570 nm, pipette and tip, Graduated cylinder 500 ml, Squirt bottle, manifold dispenser or Automatic microplate washer, polypropylene tube, weight scales, microtoise to measure the height.

B. Research Operational

Research and survey conducted after obtaining ETIC Certificate from UNAIR's Medical Department, recommendation letter from the Department of Health and Department of National Education of North Gorontalo region. After the study permit was granted by the principal of SDN 3 Dulukapa Sub Sumalata, socialization of the research was held for all parents and students in the school. Explanations and informed consent were requested and signed by parents as a sign of agreement that parents and children were willing to be examined for malaria, and if falciparum malaria was identified, the parents would allow the children and they were also willing to be the research subjects.

Measurement of food intake, screening of research subjects, and compliance of subjects to take the supplement were already performed through recall questionnaires and food frequency, characteristics of questionnaires sample and subject screening, questionnaires of compliance supplements consumption monitoring. Measurement of food intake used the Nutrisoft software.

Identification was conducted to determine the presence of Plasmodium falciparum parasite in the blood, and then there were thick drops and thin drops microscopic examinations and also the examination that utilized ICT (Immunochromatographic Test). After the identification results were obtained, the children who were identified having the infection were treated with ACT (Artemisinin Combination Therapy).

Treatment results were examined on day 14 after medicines distribution, and there was more identification done to check whether there were Plasmodium parasites or

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negative plasmodium in their blood. Children recovered from malaria were selected as research subjects according to inclusion and exclusion criteria.

All selected subjects were split into four groups randomly. The division of the group was: group with @ 20 mg / day Zinc (Zn), Fe @ 20 mg / day, Zn and Fe @ 20 mg / day simultaneously and the control group (placebo). The blood of the selected subjects was taken for around 7 ml for laboratory examination, including hemoglobin, leukocytes, hematocrit, platelets, albumin, Zn, Fe, IL-10 and IL-12 as the first research data in the 14th day after infection or before having the Zn and Fe supplements.

After two months of supplementation treatment, the blood of the subjects was re-examined for hemoglobin, leukocyte, hematocrit, platelets, albumin, level of Zn, level of Fe, and level of immune responses (IL-10, IL-12) as the data after the treatment. The obtained data were further analyzed statistically using SPSS software 17.0. Version.

C. Data Analysis

The obtained data were analyzed statistically by using one-way variant analysis (one way ANOVA) with 5% of confidence level (= 0.05). If the results have significantly different effects between treatments (FH> FT), then HA is accepted, and there will be a further test by using LSD to find out which treatment in the modulation of cytokines is significantly different or stronger.

III. RESULT

The malaria examination was conducted to 105 students of Elementary School SDN 3 Dulukapa, Sumalata Sub-district at grade II - V, aged 7-12 years old. The blood of all children was taken, and the malaria was examined microscopically and by ImmunoChromatographic Test (ICT). Out of 105 students, 79 children are positively infected by malaria falciparum. Besides, five of the children were detected to experience the growth of gametes. Positive samples counted the number of its parasites, then students who were detected with positive malaria were given a treatment with anti-malarial drugs ACT and antacids to avoid nausea as the side effect of the drugs.

After screening through the inclusion and exclusion criteria, 40 children were obtained. Those children were divided into four groups randomly; each treatment group, consisting of ten students, was differently given 20 mg of Zn, 20 mg of Fe, and 20 mg of Zn and Fe simultaneously along with the control group. The supplement was provided for eight weeks in which supplements were given each week from Monday to Saturday, so that there were 48 times in eight weeks.

There were 39 children left until the end of the study, and only one child who did not come at the time of taking the blood. All samples in each group were tested for Hb, leukocyte, thrombocyte, hematocrit, albumin, zinc, Fe and immune response measurements, i.e. IL-10 and IL-12.

➢ *IL-10 levels*

TABLE OF IL-10 LEVELS ON DAY 14 AFTER MALARIA INFECTIONS AND DAY 48 AFTER TREATMENT OF ELEMENTARY SCHOOL CHILDREN IN DULUKAPA VILLAGE

Group	Average ± SD Level IL-10 (pg/ml)		P (Sigc)
	Day-14 After Malaria Infection	Day-48 After treatment (Supplementation)	
Control	11,42 ± 10,65 [a]	0,58 ± 0,39[a]	0,011
Fe Supplement	14,26 ± 11,06 [a]	0,27 ± 0,34[a]	0,003
Zn Supplement	12,41 ± 9,01 [a]	0,45 ± 0,32 [a]	0,004
Zn and Fe Supplement	12,37 ± 10,63 [a]	0,55 ± 0,40 [a]	0,007

Table 1

The same superscript in the same column shows no difference; SD: Standard Deviation

➢ *IL-12 levels*

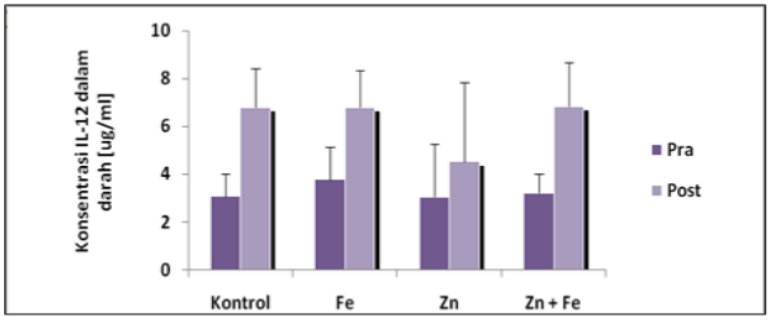

Fig 1:- IL-12 examination results; Pre: Day 14 after malaria infection; Post: Day 48 after the treatment (supplement).

IV. DISCUSSION

The results of IL-10 examination shows that in all treatment and control groups, there is a significant difference between IL-10 levels on the 14th day after malarial infection and IL-10 levels on the 48th day after supplementation. In this study, IL-10 levels in all groups experience a significant decrease. The results of the IL-12 examination reveal that the group given Zn does not show a significant difference; meanwhile, the groups provided by Fe, Zn and Fe simultaneously and the control group go through a significant difference between the IL-12 levels on the 14th day after the malaria infection and the IL-12 levels on the 48th day after supplementation.

Khanam, (2018) states that Zn may increase or inhibit immune responses so that Zn deficiency affects immune responses. Zn deficiency rapidly reduces antibodies and cell-mediated reactions in the fight against parasites and viruses in humans. A mild Zn deficiency is followed by an imbalance of Th1 and Th2 functions, causing an imbalance of endurance on transmission, so that Zn supplementation is still needed.

The results reveal that treatment groups which are given Zn supplements do not significantly increase the levels of IL-12. An approach that can describe this is that Zn supplementation is less favorable if the study subjects have the normal nutritional status of Zn (Muller et al., 2001).

Various studies on humans and animals have demonstrated the presence of cell-mediated immunity and other non-specific immunity in iron deficiency. Nevertheless, the relationship between infection and iron deficiency remains unclear. Susceptibility to infection is very complex and depends not only on Fe content but also on body, parasite and environmental factors, including, exposure to microorganisms, other nutritional deficiency factors, population types (infants, children, women, men, and parents), severity of the disease, duration of deficiency, types and doses of patients, and duration of Fe therapy and other preconditions. These factors affect susceptibility and severity of infection regardless of Fe (Lauffer, 2018).

Changes in cytokine characteristics due to stimulation of immune responses may affect the status of Fe and erythrocytes. Chronic immune stimulation is usually accompanied by a decrease in serum Fe content. Serum Fe Feat reduction is used to reduce the availability of Fe in an attempt to stop cell proliferation, reduce radical oxygen products and or prevent the use of iron for the growth of germs. The effect of cytokines on the availability of Fe is primarily mediated by the absorption of Fe in the duodenal epithelium and inhibits the release of Fe from macrophages with the aim that Fe is not readily accessible by pathogens (Koorts et al., 2011)

At the time of infection, IL-12 proinflammatory cytokines are responsible for eliminating parasites. These results should not be the same in every geographic region; IL-12 will shift in any acute conditions. IL-10 is not particularly needed to assist IL-12 in removing parasites during TNF-α and IFN-γ excessive production. If IL-12 is optimal to kill parasites through T regulators, it does not necessarily require IL-10 (Abdalla and Pasvol, 2004). It is known that an increase or decrease in IL-12 links with the disease severity. Lyke's study (2004) discovers a slight increase in IL-12 in severe malaria compared to uncomplicated one. The results of Abdalla S, Pasvol G. Platelets and blood coagulation in human malaria. In: Newton PN, Essien E, White NJ, editors. The Haematology of Malaria. London, UK: Imperial College Press; (2004) 249-276. This study finds out the lower IL-12 levels in severe malaria compared to mild malaria ($p < 0.001$). The results of this study show the lower levels of IL-12 in positive malaria infection status, but the statistical test cannot be performed due to the small number of samples. The little cause of IL-12 increase is probably because of the down-regulation by IL-10, or a combination of impaired phagocytic function due to the hemozoin consumption.

Malagnarnera et al. (2002) note that IL-18 and IL-12 rates in children with mild malaria are higher than the severely malarial children. These findings suggest that the production of these two cytokines (IL-18 and IL-12) may be corregulated and both have immunoregulatory effects on the immune response to Plasmodium falciparum infection.

The right balance between proinflammatory along with anti-inflammatory cytokines that mediate the innate and adaptive immune response is necessary for effective protection against malaria in humans and to avoid immunopathology. Macrophages release IL-12 for the first time. The IL-12 effector mechanism is very important because it has a protective effect either to kill or inhibit parasites, activate phagocytizes or increase the destruction of nitric oxide. IL-12 may also increase sequestration of adherens in parasitic vessel walls. The infected red blood cells will attach themselves to the endothelial wall.

IL-12 levels are further enhanced by IL-6 and TNF-α. So in people with increased TNF-α, the inflammatory response is weighty and is not necessarily better. As a result, it requires an inhibitor by way of feedback to prevent excessive inflammatory response by removing anti-inflammatory or anti-inflammatory cytokines, i.e. IL-10. This implies that IL-10 will block IL-12 and IFN-γ from continuing to TH1. When it goes down, it will be maintained in TH2 using TGF-β. TGF-β is an early and most noticeable cytokine response that inhibits IFN-γ. If it cannot be inhibited, hemofagositosis will occur, and the red blood cells will be damaged constantly. Normal red blood cells will be damaged and increasing adhesion molecules. If the adhesion molecule is added, the sequestration also increases so that cerebral malaria may occur (Mbugi, 2009).

There is no difference between the treatment and the control of the possibility caused by the action of the drugs given to the children who are likely to make the parasite hide or recrudescence to provide an opportunity for the

body of the children to have improved nutrition. Lubis (2008), in his study of reticulocyte counts, states that after treatment, the reticulocyte response can be seen in 48-72 hours, with a maximum response of days 5-10. The result of this study is that in the case of malaria that is classified as seasonal, with low transmittance and low API value, produces protective levels of the IL-12. Generally, in areas with low transmittance and low API values and seasonal, malaria cases often break out due to the unstable or periodic parasite exposure, so that the body is unable to stimulate the immune system. In this study, IL-12 levels are increased after the supplementation of Fe and Zn, while IL-10 is decreased. An approach that can describe the situation is that the presence of seasonal malaria, low transmissibility, low API values and abnormal Zn levels in the 9-12-year-old children group lead to increased protective IL-12 levels, so that IL-10 is no longer needed for re-stabilizing the levels of IL-12. For this reason, the levels of IL-10 in this study are decreased.

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