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Stress Family Experience And Profiles Of Tumor Necrosis Factor Alpha And Interleukin-10 Of Nuaulu Tribe Community With Hunting Activity In Mesoendemic Area Of Malaria

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ABSTRACT

The Nuaulu tribe is one of the indigenous tribes in Maluku Province, Indonesia which becomes malaria endemic area. This tribe still adheres to the customs and habits of its ancestors, one of them is the habit of hunting. It is carried out to fulfill the needs of life and the implementation of traditional rituals performed from afternoon to morning or day in the forest, depending on seasonal activities. The hunting location is in a forest that is far from the residential area. Hunters are often exposed to mosquitoes from entering the forest to collect honey. However, this is not considered a problem, because of passion in fulfilling the hunters' needs of life and stress family experience. The results of this study showed that stress family experience and the hunting group had lower profile on tumor necrosis factor (TNF- α) and interleukin-10 (IL-10) compared to the group that did not hunt and no one infected by malaria. Malaria sufferers were only found in non-hunting groups.

Keywords: Stress Family, Tumor Necrosis Factor, Interleukin-10, Malaria, Mesoendemic

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INTRODUCTION

Malaria is caused by protozoa of the genus *Plasmodium* and it is responsible for high levels of morbidity and it also has serious impact on stress family experience, socio-economic development in endemic areas. *Plasmodium vivax* is the main species that cause malaria, responsible for 80% of cases in Indonesia¹. Malaria is complex disease involving genetic factors inherent in parasites and hosts, geographical and environmental aspects². During the infection, antibody-mediated and cell-mediated immunity plays an important role in achieving clinical immunity^{3,4}. Research shows that the successful completion of malaria infection depends on the host's ability to induce adequate levels of pro-inflammatory and regulatory cytokines during the main stages of infection. Thus, the adjustment between the inflammatory and anti-inflammatory response seems to be determining factor in the clinical outcome of the disease⁵. Maluku Province is one of endemic provinces of malaria in Indonesia. The Nuaulu tribe is one of the isolated tribes in Seram Island, Central Maluku Regency, Maluku Province, Indonesia. This tribe is a tribe that still adhere to the customs of its ancestors, one of them is the habit of hunting. It is carried out to fulfill the needs of life and the needs of implementation of traditional rituals performed from afternoon to morning or even days in the forest that depends on the sustenance. The hunting location is in a forest that is far from the residential area. This habit makes the hunters in this tribe are often exposed to the mosquitoes. The public recognizes that they are very often bitten by mosquitoes from entering the forest to

going back home again to the settlement. However, this is not considered a problem since there is a passion in fulfilling the hunters' needs of life and part of stress family experience⁶.

Although it was included in malaria-endemic area, the results of the main blood survey showed that of 100 people who were examined in Nuaulu tribe community with history of fever in the last three months, there were only two people (2%) who had *Plasmodium* in their blood samples. Acquired immunity studies of malaria in endemic areas originally provide greater potential for understanding the regulation of human immune responses to malaria parasites. However, rare people is known about malaria-specific immunity acquisition in areas that are unstable, mesoendemic, or hypo-endemic transmission⁷. Several studies have focused on plasma profile of cytokines and chemokines in *vivax* malaria infection and further relationship with severity of the disease, determined by clinical symptoms, or immunological profiles after treatment with anti-malaria drugs. The overall profile of cytokine production is still contradictory due to differences in the study population and the level of endemicity in the region, and other factors which require further investigation⁸. This study aims to reveal the profile of TNF- α and IL-10 related to hunting habits in this tribe community in a malaria mesoendemic area.

RESEARCH DESIGN AND METHOD

The type of this research is an observational analytic study with cross-sectional design used to explore stress family experience explorative with questionnaire. The

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Research instrument used was Kempe Family Stress Inventory with sensitivity of KFSI was calculated at 80%. The specificity was 89.4%, the positive predictive value was 52.5%. The negative predictive value was 96.8%. The sample size was calculated using power analysis. The sample size is 1) power of a statistical test (1-β). 2) level of significance (α), and population effect size (γ). Therefore, the sample size in this study has estimated the significance at 0.05 a power of 0.80 and the small effect size 0.25 requiring a sample size of 84 families for the study. The questionnaire of this study was developed by the researcher based on a literature review of Kempe Family Stress Inventory^{7,8,9,10,11,12,13,14}. The instruments included 10 items: 1) Family was Beaten or Deprived. 2) Criminal or Mental Illness Record or Substance Abuse History. 3) Family Suspected of Abuse in the Past. 4) Family with Isolation, Low Self-esteem, or Depression. 5) Multiple Stresses or Crises. 6) Violent Temper Outbursts. 7) Rigid, Unrealistic Expectation. 8) Harsh Punishment. 9) Child is being felt difficult to perceived by family. 10) Unwanted or at Risk for Poor Bonding. The Kempe Family Stress Inventory consisted of 10 items third degrees were 1) Normal. Mild (rate as 5 if one or more applies). Severe (rate as 10 if one or more applies). The sensitivity of the KFSI was calculated at 80%. The specificity was 89.4%. The positive predictive value was 52.5%. The negative predictive value was 96.8%^{15,16,17,18}. After that to explore determine the profile of TNF α and IL-10 in the Nuauulu Tribe community with hunting and non-hunting habits. The research areas were Rohua Hamlet and Bonara Hamlet, Sepa Village, Amahai Subdistrict, Central Maluku Regency. The research population was the indigenous people of the Nuauulu tribe who lived in the research areas. The sample was part of the study population that met inclusion criteria, i.e. people aged 18 to 60 years, living in the research area for at least last 3 years, and was willing to be the research respondents. Age limitation was meant by considering that the ages of 18 to 60 are adults who often carry out hunting activities. The samples were

divided into two groups, i.e. hunting group consisting of 40 people and a non-hunting group consisting of 44 people. The sample was determined using simple random sampling. Blood plasma research samples were used to examine TNFα and IL-10. The blood samples were taken for 3 cc, put in the Vacutainer® blood collection tubes, and then stored in a portable blood cooler filled with an ice pack at -20°C. They were immediately brought to be centrifuged at speeds of 3000 rpm for 15-20 minutes to get the plasma. The plasma layer then transferred to 1.5-micron Eppendorf tube, put in an Eppendorf tube box, and stored at -800 °C. Legendmax™ ELISA Kit Human TNF α and IL-10 from Biologend, which applied a solid-phase enzyme amplified Sensitivity Immunoassay method on a microtiter plate, was used for reagents examination Quality. The guarantee of quality control was carried out by looking for the inaccuracy of samples examined within run and between run. A malaria examination was carried out through microscopic examination using Giemsa. To test the differences in levels of TNF α and IL-10 among the research samples, the normality test was first conducted using Kolmogorov-Smirnov test. The results of normality test of TNF α and IL-10 levels in hunting group were p = 0.47 and p = 0.48, while in non-hunting group the results of normality test for both cytokines were same (p = 0.00). Based on the results of normality test in hunting group, the distribution of data was normal, while those who do not hunt showed that the distribution of data was not normal so that the test of differentiation was a non-parametric test using the Mann-Whitney test. The normality test of TNF α and IL-10 levels based on malaria and non-malaria obtained a value of p = 0.539, p = 0.210 and both showed normal data distribution. Statistical analysis of the present research used IBM SPSS statistical version 21 software.

RESULTS

1. Tabel 1. Distribution of Demographic data

Characteristics of family N (84)	Hunting (N=40)	Non-hunting (N=44)
	N =%	N =%
Age (Years)		
18-25	16 (40)	24 (55)
26-60	24 (60)	20 (45)
Education		
Primary School	23 (58)	23 (56)
Junior high schools	13 (33)	13 (33)
Senior high schools	4 (9)	4 (11)
Relationships: the family of patient		
Couples	31 (78)	31 (78)
Parents	6 (15)	6 (15)
Brothers/sisters	3 (7)	3 (7)

2. Tabel. 1 Overview Stress Family Experience with hunting and non-hunting habits

Stress Reaction	Hunting	Non-hunting
	Mean±SD	Mean±SD
Normal	3.28 ±3.2	21.28 ±3.6
Mild	4.43 ±4.7	3.43 ±2.7
Severe	25.82 ±5.45	2.85 ±5.15
Was Beaten or Deprived	4.5±3.12	4.5±3.12
Family has a Criminal or Mental Illness Record or Substance Abuse History	8.14±2.7	5.11±2.1
Family Suspected of Abuse in the Past	22.7±1.7	4.7±1.6

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Family with Isolation, Low Self-esteem or Depression	12.4±4.23	3.4±2.23
Multiple Stresses or Crises	45.4±2.1	15.4±2.4
Violent Temper Outbursts	7.2±4.5	3.2±4.5
Rigid Unrealistic Expectation	9.1±2.3	4.1±2.3
Harsh Punishment	11.3±1.4	2.3±1.4
Being felt difficult to perceived by family	41.3±4.2	2.3±4.1
Unwanted or at Risk for Poor Bonding	8.4±1.1	3.4±1.5

Table 2 revealed that Stress Family Experience with hunting is more severe than non-hunting habits. Stress reaction Family Experience with hunting severe score was 25.82 (SD=5.45). Family Suspected of Abuse in the Past 22.7 (SD=1.7), Family with Isolation, Low Self-esteem or Depression score was 12.4 (SD=4.23). Being felt difficult to perceived by family 41.3 (SD=4.2), while for Stress reaction Family Experience with Non-hunting severe score was 2.85 (SD=5.15). Family Suspected of Abuse in the Past 4.7 (SD=1.6), Family with Isolation, Low Self-esteem or Depression score was 3.4 (SD=2.23). Being felt difficult to perceived by family 2.3 (SD=4.1),

Microscopic examination of malaria

The results of microscopic examination of malaria using Giemsa showed none of hunting group (0%) was infected with plasmodium in their blood samples, whereas in non-hunting group showed that three samples out (7%) of 44 samples were found to be positive for Plasmodium in their blood samples. The plasmodium found in these three people were *p.vivax*.

Table. 1. Results of Microscopic Examination of Malaria-based Samples Distribution

Laboratory results	Behavior				Σ
	Hunting (n=40)		Non-Hunting (n=44)		
	Σ	%	Σ	%	
Negative	40	100	41	93	81
Positive	0	0	3	7	3

Source: Primary processed data, 2018
Levels of TNF α and IL-10

The results of TNF α examination and IL-10 levels in hunting group showed that the average levels of TNF α was 5.27 ml/pg with standard deviation of 2.42 ml/pg and mean IL-10 was 5.02 ml/pg with standard deviation of 2.12 ml/pg. Meanwhile, in the non-hunting group, the average levels of TNF α were 15.87 ml/pg with the standard deviation of 30.36 ml/pg and the mean levels of IL-10 were 14.13 ml/pg with the standard deviation of 26.59 ml/pg. Based on these data, it can be concluded

that there were differences in mean and standard deviation values on these two groups, wherein the levels of TNF α and IL-10 in non-hunting group were higher than both levels in hunting group. Differentiation tests using Mann-Whitney test showed that there were statistically different levels of TNF α between hunting group and non-hunting group (p = 0.005). On the other hand, the results of statistical tests indicated no differences in mean levels of IL-10 between hunting group and non-hunting group (p = 0.154).

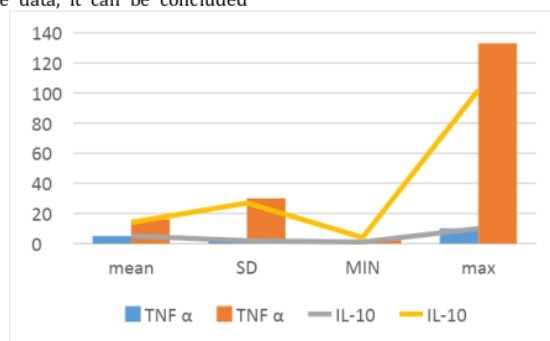


Figure 1: Comparison of hunting behavior-based mean, SD, and min-max levels of TNF α and IL-10. Differences in mean levels of TNF α and IL-10 as well as their correlation with the malaria cases. The differences in the examination results of the levels of TNF α and IL-10 based on malaria incidence in non-hunting group found that the average levels of TNF α in positive malaria by samples were 123.11 ml/pg with standard deviation of 12.01 and mean levels of IL-10 were 97.50 ml/pg with standard deviation of 14.07.

Whereas the average levels of TNF α in negative malaria by samples were 8.02 ml/pg and mean levels of IL-10 were 8.03 ml/pg. The mean levels of TNF α in non-malaria patients were 8.02 ml/pg, while positive ones were 123.11 or a 15-fold increase compared to those without malaria. The mean levels of IL-10 in positive ones were 97.5 ml/pg and those without malaria was 8.03 ml/pg or a 12-fold increase compared to those without malaria. The results of statistical test using an independent t-test found that p-value of TNF α and IL-10

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is same (0.004). Therefore, there are differences in mean values of TNF α and IL-10 between positive malaria-by samples and negative malaria-by samples. Correlation test between hunting habits with levels of both cytokines using Spearman test showed significant correlation between hunting activities and levels of TNF α ($p < 0.03$) with correlation coefficient (r) = 0.3, but there was no significant relationship between hunting activities and levels of IL -10 ($p > 0.05$). The correlation test between levels of TNF α and IL-10 using Spearman test also obtained significant relationship between levels of TNF α and levels of IL-10 ($p = 0.000$) with correlation coefficient = 0.4.

DISCUSSION

The present study assessed for the first time Stress Family Experience with hunting is more severe than non-hunting habits. Stress reaction Family Experience with **hunting severe stress reaction, Family Suspected of Abuse in the Past, Family with Isolation, Low Self-esteem or Depression, Being felt difficult to perceived by family.** However, this is not considered a problem since there is a passion in fulfilling the hunters' needs of life and part of stress family experience^{16,17,18}

Annual Parasitic incidence (API) data obtained from Puskesmas or Public Health Center and the results of an examination of the enlarged spleen in students indicated that the residential areas of Nuauulu tribe were included in malaria mesoendemic area. It is the area where malaria does not occur throughout the year, but only in certain months (unstable transmission). However, if an extraordinary event happens it is estimated that 10-15% of deaths from malaria will be able to occur. Hunting becomes one culture of Nuauulu people who are at risk of malaria infection which is still maintained today. Hunting activities are carried out to fulfill the daily needs of life and the part of traditional rituals according to the community's trust, which according to Ellen is classified as animism. Hunting activities are carried out from late afternoon until tomorrow morning or depending on the sustenance obtained^{19,20}. Being exposed to mosquitoes in the forest is common since they are always bitten by mosquitoes from entering the forest until going home. However, this is considered normal because they think that there is something more crucial than being bitten by mosquitoes, i.e. to fulfill the needs of their families and other people²⁰.

The results confirmed that mean levels of TNF α and IL-10 in hunting group were lower than those in non-hunting group. After being statistically tested, there were differences in mean levels of TNF α between the two groups. The incidence of malaria only occurred in groups where people did not hunt. On the other hand, there were no malaria sufferers in hunting group. The correlation test showed a significant relationship between levels of TNF α and IL-10 with the incidence of malaria²¹.

Infection may occur if the first defense (innate immunity) cannot block the invasion of pathogens to the body^{22,23}. The immune response is very dependent on the ability of immune system to recognize foreign molecules (antigens) contained in potential pathogens and then generate the right reaction in getting rid of the source of antigen. Clinical manifestations of malaria, including hematological changes, have been associated with several factors, such as demographic characteristics, the levels of malaria endemicity nutritional status, and malaria

immunity^{24,25,26}. The immune system is a defense mechanism that can form a wall of immunity to restrict infection cells and molecules that are responsible for immunity to build body immune system, as well as the collective and coordinated response of the immune system to recognize foreign substances, is called an immune response^{27,28}

If the immune system is exposed to substances that are considered as foreign, then there are two types of immune responses that may occur in the form of innate and adaptive immunities response. These two most important systems among integrated activities include: 1) The innate immune response stimulates and affects nature of adaptive immune responses; 2) The adaptive immune system is obtained by using various effector mechanisms of the innate system to get rid of pathogens and often improve innate immunity function. These interactions take place together in an integrated manner in such a way as to produce a biological activity that is in rhythm and harmony like a concert. At the beginning of the immune response initiation, a group of functional cells called antigen-presenting cells (APC) capture the antigen then present it to the lymphocytes in form of known by lymphocytes. The main professional APC that has been known is macrophages. Besides, those that may function as APC are dendritic cells, B cells, astrocytes, endothelium, and fibroblasts. Dendritic cells are also known as cells that connect between innate and adaptive immunity.²⁹

Different ways of presenting determine if an immune response will occur and which type of immune response will occur. Innate immunity does not only serve to provide an early response to the pathogens but also plays an important role in inducing adaptive immune responses. The collaboration between the initial congenital and subsequent adaptive responses is needed for the effective functioning of immune system and immune responses. Innate immunity has an important role as the first line of defense against pathogens and in development of adaptive immune responses. If the sporozoites are injected into skin by biting mosquitoes, it will flow to the lymph nodes, the main ripening sites of T and B cells, or to the liver to attack hepatocytes. Antibodies (Ab) trap sporozoites in skin or prevent the invasion of liver cells. T cells produce IFN- γ , CD4 +, and CD8 + to inhibit the development of parasites into merozoites in hepatocytes (5). IFN- γ producing lymphocytes activate macrophages and increase merozoite phagocytosis then infected red blood cells that are not consolidated³⁰

Generally, malaria is characterized by TNF α expression that occurs in acute phase yet in small numbers. Meanwhile, IL-10 is produced after prolonged exposure. The increased production of TNF α which elevates with the increasing IL-10 may indicate the presence of balance cytokines because if the production of TNF α is higher, it will not only used to suppress pathogen replication but also can be used to damage the body's organs^{28,29,30}. The ratio of two cytokines also illustrates the pathogenesis of populations in malaria-endemic areas and may give an idea of cytokines number in acute period and times of convulsions may occur^{30,31}. The plasmodium examination showed that from 84 people of the research objects, there were only three people who were positive for malaria, indicating that the immune system was good because

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they lived in an endemic area where malaria can infect them, but malaria cases were low there.³²

The parameters that can modulate immune responses include nutritional status, changes in circulating cytokine levels, expression of adhesion molecules, chemotaxis or mobility changes, and generation of reactive species. The immune response is also influenced by factors such as age, gender, biological rhythm, and lifestyle³². The other activities that Nuauulu people have done during their hunting activity in the forest like; food gatherings, exercises or physical activities, the use of herbs to prevent fever, and periodically being bitten by mosquitoes make them stronger than usual to prevent their bodies from malaria.

CONCLUSION

The profiles of TNF α and IL-10 in hunting groups are better than the average values in non-hunting groups. The tradition of hunting in Nuauulu people has caused the individuals being immune or resistant to malaria, even it had a positive impact on their health. This is because there were behaviors of food gathering and consumption of various food which fulfill good nutrients for their bodies during hunting activities. Also, it becomes a training activity that has certain impacts on their immune system so that it can prevent the body from disease. Besides, the use of herbs, local wisdom, and regular exposure to disease vectors has an impact on the activation of immune system function. Unconsciously, the tradition of hunting in Nuauulu tribe has a positive relationship with their health status during hunting activities.

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