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Analysis of Interleukin-4 Level in Patients with Autoimmune Thrombocytopenia

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ABSTRACT

Thrombocytopenia is a disease characterized by a decreased platelet count. Some of the causes are decreased platelet production, increased platelet use, such as due to infection, and autoimmune causes, namely the loss of tolerance of the immune system to self-antigens on the surface of the platelets and megakaryocytes marked with a platelet count <100,000/µL. Based on the pathomechanism, this disease is classified into primary ITP, non-primary ITP, and ITP caused by other conditions. IL-4, one of the cytokines produced by Th2, stimulates B cells to increase antibody production. This study aimed at comparing the IL-4 levels in primary ITP patients and non-primary ITP patients. This study involved 30 primary ITP subjects and 30 non-primary ITP subjects obtained based on data from medical records through IL-4 cytokine level examination using the ELISA method. The results of this study revealed that the IL-4 levels of the non-primary ITP subjects were higher than the primary ITP subjects, which signifies differences in IL-4 levels between both groups.

Keywords:
primary ITP; non-primary ITP; IL-4

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INTRODUCTION

Thrombocytopenia is a condition in which the number of platelets in the body decreases from the normal number, which is <150,000 - 450,000/µl of blood. This condition is attributed to decreased platelet production due to bone marrow damage, myelofibrosis, myelodysplastic syndromes, and genetic defects. Thrombocytopenia is also caused by increased platelet consumption due to autoimmunity (Matzdorff et al., 2018). Based on the pathomechanism, autoimmune thrombocytopenia consists of primary immune thrombocytopenia (ITP), which is characterized by a platelet count of less than 100,000 µL (Sari T, 2018). Non-primary ITP is caused by primary diseases, including infections (such as Helicobacter pylori, Human Immunodeficiency Virus/HIV, Hepatitis C Virus/HCV, etc.), autoimmune disease (such as Systemic Lupus Erythematosus/SLE, Rheumatoid Arthritis/RA, thyroid disease, Antiphospholipid Antibody Syndrome/APS, Evans syndrome/ES) (Zufferey et al., 2017), lymphoproliferative disease (chronic lymphocytic leukemia/CLL and granular lymphocytic leukemia/LGL) and certain drugs (Cines et al, 2009). Moreover, thrombocytopenia can happen due to splenomegaly, bleeding, and severe infection (sepsis) (Matzdorff et al., 2018).

Primary ITP is an autoimmune disease caused by the destruction of normal platelets due to the presence of autoantibodies (antibody-mediated destruction of platelets) and impaired production of megakaryocytes. This disease is a disorder attributed to immune dysregulation with the end result of the loss of the immune system tolerance to self-antigens that are on the surface of platelets and megakaryocytes. T cells are activated due to platelet-specific antigen recognition on APC (Antigen Presenting Cell), which then induces antigen-specific expansion in B cells. Then, B cells produce autoantibodies specific to glycoproteins expressed on platelets and megakaryocytes. The circulating platelets are bound by platelet autoantibodies and adhesion to the spleen macrophage FC receptor happens, destroying platelets. Also, anti-megakaryocyte autoantibodies are formed, which reduce the ability of megakaryocytes to produce platelets (Sari Teny Tjitra, 2018). Although the pathogenesis remains unclear, it is believed that primary ITP is resulted from the development of IgG autoantibodies that target structural platelet membrane glycoproteins IIb-IIIa (Zainal et al, 2019).

Based on data from the International World Health Organization (WHO), the prevalence rate of ITP is increasing from year to year. The prevalence rates of ITP in a number of countries were 1.8 cases/1000 deliveries in Finland, 0.5-1.5 cases/1000 deliveries in the UK and France, and 80% of cases of FNAIT (Fetal Neonatal Alloimmune Thrombocytopenia) in Japan due to HPA (Human Platelet Antigen) incompatibilities). The prevalence rate of ITP among adults in the US was estimated at 3.3/100,000/year, the prevalence in children was estimated at 5.3/100,000/year (the number of ITP in children was diagnosed almost the same every year), and the prevalence of cases was 9.5/100,000/year. The ratio of men and women is almost balanced, with 52% of men and 48% of women. About 40% of all patients diagnosed with ITP were children under 10 years old, most commonly diagnosed at 2 to 4 years of age (NORD, 2015).

Another study from France reported an overall incidence of ITP of 2.92/100,000/year. The prevalence rate in women (3.03/100,000/year) was higher than in men (2.77/100,000/year). Meanwhile, incidence of non-primary ITP occurred with various conditions, such as malignant lymphoid disorders (5.9%), SLE (2.5%), myelodysplastic syndrome (2,3%), immune deficiency (1.7%, excluding HIV infection), HIV infection (0.9%), sarcoidosis (0.6%), antiphospholipid syndrome (0.3%), and HCV infection (0.2%), as well as adult patients (1.63%) and children (1.1%) with Evans syndrome (Moulis et al., 2014).

Cytokines are molecules that are responsible for controlling intracellular communication and directing immunological reactions (Abbas, 2016). Cytokine balance regulates the immune system under normal and disturbed conditions in many autoimmune diseases. Several studies support the role of serum cytokines in the pathogenesis of ITP and provide evidence showing that polarized T-helper lymphocytes into Th1 and Th2 immune responses lead to B cell differentiation with the production of autoreactive antibodies in ITP (Pavelić, 2013. Differentiation of naïve T-helper cells into Th2 cells is stimulated by IL-4, which may be produced by mast cells, other tissue cells, and T cells themselves. Th2 cells also produce more IL-4 which stimulates B cells to increase antibody production (Abbas Ak, 2016).

Several studies showed that IL-4 contributed to the pathogenesis of ITP as evidenced by an increase in serum IL-4 levels and the presence of IL-4 gene polymorphisms in ITP patients compared to healthy control. Research Srdana Culic, et al. (2013) explained that the serum IL-4 levels of ITP patients were higher than that of healthy control. The study by Manal Mohamed M and Samah Mohamed AE (2014) examined the IL4 and IL10 gene polymorphisms in 70 ITP patients and 50 healthy controls. It was found that IL4 RP2 and IL10 A alleles were detected more frequently happened among ITP patients compared to controls. Combined polymorphisms of IL4 and IL10 genes are associated with a greater risk of ITP (Makhlouf & Elhamid, 2014).

Ma et al. (2014) measured the concentrations of plasma cytokines associated with Th1 (IFN-γ, IL-2), Th2-related cytokines (IL-4, IL-10), Th17-related cytokines (IL-17), and cytokines associated with Treg (TGF- β1) in 52 adult ITP patients and 30 healthy controls, evaluated using the ELISA (Enzyme-Linked Immunosorbent Assay) assay method. The results showed that the concentrations of Th2 cytokines (IL-4 and IL-10) were significantly higher in ITP patients compared to in the controls.

A recent study by Noriyuki Takahashi, et al. (2017) evaluated the impact of Th1/Th2 cytokine receptor functional polymorphisms in 126 patients with chronic Immune Thrombocytopenia (cITP) and 202 healthy controls. The results showed that cytokine polymorphisms affected Th1/Th2 and increased the susceptibility and severity to cITP.

Based on the background description, IL-4 contributes to the pathogenesis of ITP as evidenced by an increase in serum IL-4 levels and the presence of IL-4 gene polymorphisms in patients with ITP. In this study, the researchers compared the levels of IL-4 in the serum of patients with primary ITP and non-primary ITP.

MATERIALS AND METHODS

Research procedure

This research was conducted from August to December 2019 in HUM-RC Laboratory of Hasanuddin University Hospital and the research samples were taken from Dr. Wahidin Sudirohusodo Central Public Hospital in Makassar.

A total of 60 samples were used in this research, with 30 subjects of primary ITP thrombocytopenia and 30 subjects of non-primary ITP, who had routine blood tests and showed a platelet count <100,000/µl. The subjects were officially diagnosed and proven from the medical record data. The samples were gathered using a purposive sampling technique. The IL-4 examination was performed using the ELISA method.

Data Analysis

The data on the characteristics of the research subjects were presented descriptively. To determine the data distribution, the Shapiro-Wilk test was performed. Since the data were not normally distributed, the Mann-Whitney test was conducted.

RESULT AND DISCUSSION

This research involved 30 subjects of primary ITP and 30 subjects of non-primary ITP, aged two to 79 years old. The characteristics of the subjects are presented in Table 1. In terms of sex, the subjects of primary ITP were 24 women and six men, and this is in line with the result of the study by Emmanuel Andrès (2016) in France, primary ITP commonly occurred in women with the ratio of 3-4:1, compared to men. This is attributed to sex hormones, which can play a role in susceptibility to ITP. Apart from having an impact on the immune system, sex hormones can also change the clinical features and response to therapy, as in some experimental animals, estrogen promotes a B-cell mediated autoimmune disease, while androgen is the opposite (Andrès, 2016). The subjects of non-primary ITP subjects in this study consisted of 12 women and 18 men.

The analysis on the characteristics of research subjects, it was revealed that most primary ITP subjects (73.3%) were aged 18-65 years. The prevalence rate was different from that was found by Sajedeh Saeidi, et.al. (2014), that among the total thrombocytopenia patients, 223 patients (69%) were diagnosed since they were childhood, with a mean age of 3.6 years, and 100 patients (31%) were diagnosed since adult, with a mean age 34.3 years.

The comparison of IL-4 levels in primary ITP subjects and non-primary ITP subjects

To investigate the comparison of IL-4 levels in primary ITP subjects and non-primary ITP subjects, the normality test on the samples was conducted. The results showed that all groups were not normally distributed; and therefore, a non-parametric test with the Mann-Whitney test was performed. The results of the test are presented in Table 2.

Table 1. The characteristics of the research subjects

Characteristics		Primay ITP (n=30)		Non-primary ITP (n=30)	
		N	**%**	**N**	**%**
Sex	Male	24	80	12	40
	Female	6	20	18	60
Age	2 - 17	4	13.3	4	13.3
	18 - 65	22	73.3	21	70
	66 - 79	4	13.3	5	16.7
Classification of thrombocytopenia	a. PLT Production ↓	-	-	13	43.3
	b. PLT Consumption ↑				
	• Primary ITP	30	-	-	-
	• Non-primary ITP	-	100	12	40
	c. Other types of thrombocytopenia	-	-	5	16.6
Average thrombocytes (10^3/µl)		40.7		49.23	
IL-4 (ng/L		26.1		29.5	

Table 2. the comparison of IL-4 levels in primary ITP subjects and non-primary ITP subjects

Subject	N	Interleukin 4 (ng/L)		*p
		Range	Median	
Primary ITP	30	12-75	19.5	0.003
Non-primary ITP	30	15-1054	29.5	

*P: Mann-Whitney test

The results of the statistical test exposed that the median of IL-4 level in primary ITP subjects was 19.5 ng/L, while the median of non-primary ITP subjects reached 29.5 ng/L, with the *p*-value =0,003<α=0,05. In other words, non-primary ITP subjects had a higher median than primary ITP subjects, with a significant difference. This is associated with the diseases causing non-primary ITP that contribute to a higher increase in IL-4 level.

Diseases that trigger non-primary ITP were found in samples with a diagnosis of infection involving B cell activation. B cell activation and differentiation produced IL-4-induced antibodies. IL-4, a mediator that is one of the most frequently associated cytokines in inflammatory disease, is mainly produced by activated T helper 2 (Th2) cells although mast cells, basophils, and eosinophils are also known to secrete IL-4. (Abbas Ak, 2016). One of the causes of infection is HCV, a virus transmitted through the blood that reaches the liver through blood circulation. After entering the liver, this virus replicates in the liver cells (Kistangari & McCrae, 2013). The study by Zhitong Wu, et al. (2015) reported that IL-4 polymorphisms were associated with an increased risk of HCV infection and hepatocellular carcinoma, especially among Asian populations. This study also included the samples of aplastic anemia, which is a disease of bone marrow hematopoietic tissue damage and results in excessive apoptosis of hematopoietic cells. Hong-Xia Zhang (2016) examined the changes and significance of Th1/Th2 in patients with aplastic anemia, with results depicting that Th1/Th2 cells in the observation group were significantly higher than in the control group. This study also reported that Th1/Th2-related cytokines, namely IFN-γ/IL-4, in the observation group were significantly higher than in the control group. Severe sepsis is also a diagnosis that commonly occurs in non-primary ITP subjects. Schulte et al. (2013) reported that IL-4 mRNA expression was associated with survival in patients with severe sepsis. This study suggested that IL-4 plays an important role in the pathogenesis of sepsis, even though the exact role in the disease has not been identified.

CONCLUSION

This study concludes that the IL-4 level of subjects with non-primary ITP was higher than that of the subjects with primary ITP, indicating a difference in the IL-4 level between both groups.

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