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Number of Lymphocyte B Cells and Level of Immunoglobulin G in Down Syndrome Patients in Indonesia

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ABSTRACT

The purpose of this study is to measure the amount of lymphocyte B cells and the level of Immunoglobulin G (IgG) as an indicator of infection susceptibility in DS patients. This research used case-control design. Samples were 20 DS patients, and 25 control non-DS, from 4 to 16 years old. DS patients and non-DS were identified by physical examination and chromosome examination. Venous blood was taken from each subject for chromosome examination, counting the number of lymphocyte B cells CD19+ and measuring IgG levels. Data were analyzed by Mann-Whitney test. This study found that the average number of lymphocyte B cells CD19+ in patients with DS was 305.05 ± 162.52, which was lower than the control group (750 ± 501.74) with p=0.00. The mean IgG levels in DS patients (1421.20 ± 363.37) were found to be higher than the control group (1291.80 ± 91.69), p>0.05. This study showed that the number of lymphocyte B cells CD19+ was lower in DS patients than controls. It may be due to the continuous activation of the immune system that will lead to apoptosis resulting in a decrease in the number of lymphocyte B cells CD19+.

1. Introduction

Susceptibility of infection can lead to a lower health expectancy of Down Syndrome (DS) patients [1]. This infection can also occur in people with DS and exacerbated with impaired immunity [2- 4]. Immunity disorders cause DS sufferers often experience respiratory tract, gastrointestinal tract, and ear infections [2], [3], [5]. Pneumonia is the most common respiratory tract infection found in DS patients and the first leading cause of death [3], [4]. Pneumonia and bronchopneumonia are the cause of the high risk of death in patients with DS [6]. In addition, respiratory syncytial virus (RSV) infection also has a higher risk of death in DS patients. This may be due to a lower immune factor in DS patients [4], [7], [8].

Disorders of the immune system in people with DS is due to a deficiency or dysfunction of the specific and

nonspecific immune system. Some researchers have found that there is an increase in Natural Killer cells (NK cells), but the activity of these NK cells is lower than healthy people. Besides, it is also reported that the activity of neutrophils and leukocytes decrease due to impaired ability of phagocytosis and bacteriocide [4], [9], [10]. Disorders of the immune system in these patients is also associated with a decrease in the number of lymphocytes T and B cells in the blood, reduced, and the low response of specific antibodies. Lymphocyte B cells are particular immunities that play a role in producing antibodies or immunoglobulins to attack microorganisms, especially bacteria. Disorders of lymphocyte B cells increase the risk of the respiratory tract and gastrointestinal tract infection, sepsis, and meningitis [3], [4], [11- 13].

Deficiency of lymphocyte B cells transition and naïve in DS patients may be due to an increase of apoptotic processes, the failure of lymphocyte cell production, and cleavage disorders. Lymphocyte T cell deficiency may also affect the activation of lymphocyte B cells [11]. This immune cell disorder is one of the causes of susceptibility to infection in DS patients. The abnormality of lymphocyte B cells in DS patients over six years old tends to increase serum immunoglobulin G (IgG) and immunoglobulin A (IgA) and decrease immunoglobulin M (IgM). This increases respiratory tract infection in DS patients. Immunoglobulin G is the most widely produced antibody and has the most prolonged half-life [2- 4].

Respiratory tract infection of DS sufferers in Indonesia is probably caused by a bacterial infection. This may be due to lymphocyte B cell disorders in stimulating the formation of antibodies or immunoglobulins. However, to date, there has been no research in Indonesia about the number of lymphocyte B cells and IgG levels in patients with DS. The surface marker for B peripheral lymphocytes is CD19+. This study aims to determine the number of lymphocyte B cells CD19+ and IgG levels as an indicator of infection susceptibility in patients with DS.

Research design

This study was observational research by using a cross-sectional design. The number of samples was 20 people with DS and 25 control (non-DS people) in Educational Hospital Bandung Indonesia with the age range 4-16 years. There were no differences in gender and age between the DS group and control. This research has passed the research ethics code at Faculty of Medicine Andalas University No: 082 / KEP / FK / 2015.

Examination methods

Venous blood was taken seven ccs each sample for chromosomal, lymphocyte B cells, and IgG level examination. Karyotyping examination was conducted in the laboratory of the Faculty of Medicine Padjadjaran University using three stages, i.e., planting, harvesting and painting, and analyzing. The number of lymphocyte B cells was counted by the flow cytometry method using the BD Facs Calibur tool in Dharmais Hospital. Reagent cd3 / cd19 / cd45 was used to examine the peripheral marker of lymphocyte B cells, which was CD19+. The examination of IgG levels by PEG-enhanced Immunoturbidimetric method was performed in clinical laboratory Prodia.

Statistical analysis

The data was analyzed by using Mann-Whitney Test to see the difference of lymphocyte B cells count and IgG levels between the two groups.

2. Findings and Discussion

Differences in the number of lymphocyte cells CD19+ and mean IgG levels between people with DS and control can be seen in table 1 and table 2. This study found that the number of lymphocyte B cells CD19+

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on DS patients was 305.05 ± 162,52 (107 – 651) cells/µl, and the control group was 750.00 ± 501.74 (166 – 2533) cells/µl. The number of lymphocyte B cells CD19+ in the DS group was lower than the control group and statistically significant with p-value = 0.000 ($p < 0.05$).

Table 1. The differences average of the number of lymphocyte B cells CD19+ in the DS and control group

Variables	Groups		p-value
	Down Syndrome (n=20)	Control (n= 25)	
Number of lymphocyte B cells $CD19^+$ (cells/µl)	305.05 ± 162.52	750.00 ± 501.74	0.000

Table 2. The differences mean of IgG levels in the DS and control group

Variables	Groups		p-value
	Down Syndrome (n=20)	Control (n= 25)	
IgG Levels (mg/dl)	1421.20 ± 363.37	1291.80 ± 291.69	0.258

The mean IgG level in DS patients was 1421.20 ± 363.37 (827 - 1904) mg/dl, which was higher than control group (1291.80 ± 291.69 (873 - 2007) mg/dl). However, the difference was not statistically significant with p value 0.258 ($p > 0.05$). This study found that in DS patients aged 4-16 years, the number of B lymphocytes in venous blood was lower than the control group. This result is supported by a previous study reporting that people over six years old with DS are often found with abnormal lymphocyte B cells. Another study showed that the number of lymphocyte B cells in fetuses with DS was lower than the normal fetus [14]. The decrease in the number of lymphocyte B cells transition and naïf is one of the causes of impaired immunity in DS patients. Some researchers concluded that this decrease in the cell count might be due to increased apoptotic processes, the failure of lymphocyte cell formation and cleavage disorders. Lymphocyte T cell deficiency also plays a role in decreasing lymphocyte B cells activation [3], [4], [11].

Decreased activity of lymphocyte B cells can be caused by a deficiency of lymphocyte T cells. Lymphocyte T cells disrupted maturation is due to rapid apoptosis occurs in the thymus that would affect lymphocytes B cells. Some researchers suggested that the decreased number of lymphocytes T cells CD4+ in patients with DS indicates the possibility of disruptions in the thymus cells. Abnormality in thymus cells is due to an increase of SOD1 gene expression in patients with DS, causing apoptosis of thymus cells more quickly [4], [10], [15]. It is found a decrease in the total number of lymphocytes, T lymphocytes, and Regulatory T cells in children with DS. Its causes increased susceptibility to lower respiratory tract infections. Thus, the decrease in the number of lymphocytes B cells in DS sufferers may be due to disrupted maturation of lymphocyte T cells as a result of earlier thymic cell apoptosis [16].

Respiratory tract infections are most common in people with DS; 51.5% is lower respiratory tract infection, and 19.1% is the infection of the upper respiratory tract. Most often are Streptococcus pneumonia, Staphylococcus aureus, and Haemophilus influenza. Recurrent infections occurring in DS sufferers were reported due to lymphocyte B cell disorders. The infections may be due to the low level of immunity specific in DS patients. Laboratory results of adult DS patients with a diagnosis of pneumonia showed IgG deficiency. It may be due to abnormalities of lymphocyte B cells, resulting in antibody deficiency. Lymphocyte B cells are specific immune cells that stimulate the formation of antibodies, especially in bacterial infections [4], [6], [10], [13], [17], [18].

This study also found that IgG mean levels in DS patients were higher than controls, although the differences were not significant statistically. Some studies reported that there was an increase of IgG and

IgA levels and a decrease in IgM levels in patients with DS (13-19). Increased levels of IgG in patients with DS may be caused by the decline in the ability of cellular immune cells in fighting the antigen cause of infection. Impaired immune cells are evidenced by the decreased number and activity of neutrophils and leukocytes, as well as impaired ability of phagocytosis and bacteriocide. The slow response to the antigen will lead to stimulation of the immune system and increase the production of antibodies (IgG and IgA). The increase of IgG levels is more likely due to the adaptation mechanism of the body to antigens as a result of the low resistance of the cellular immune system. However, some studies found normal IgG levels in patients with DS [4], [9], [10], [18].

This study showed that statistically, there was an association between lymphocyte B cell CD19+ count and IgG levels. But the results obtained differ from the theory that the decrease in the number of lymphocyte B cells will cause a reduction in IgG levels, whereas, in this study, there was an increase in IgG levels. Increased levels of patient IgG have been described previously. This possibility occurs because of a down-regulator mechanism, in which the increase in IgG that occurs early is due to body compensation for the decline of immune cells. When the body can no longer compensate, it will decrease IgG and IgA. It is proven that increasing age of DS patients will cause a decrease in IgA levels. The same may also be true for IgG levels. Some studies also found that in fetuses and children, there is an increased level of IgG and IgA despite a decline in lymphocyte B cells [13], [14], [19], [21].

These immune cell disorders may increase the risk of infection in DS patients. This condition leads to high rates of lower respiratory tract infections, high risk of recurrent infections, the frequent presence of patients in the hospital, and an increase of hospital length of stay. Therefore, it is necessary to consider prophylactic treatment, especially for people with DS who are less than one year old [19], [22]. The susceptibility of infection in DS patients can also be caused by non-immunity disorders such as anatomical structural malformation, such as abnormalities in the respiratory tract, ear and mouth. This disorder makes it easier for microorganisms to enter the body [4]. Another factor is related to nutrition. Nutritional deficiency, especially zinc, will lead to disruption of the thymus cells. Zinc deficiency can cause faster thymus cell atrophy and T cell impaired maturation. These will lead to impaired immunity. [19] also reported that there is a link between malnutrition and immunoglobulin deficiency in DS. Early nutrition interventions will have a good impact on the quality of life of DS patients. It is proven that zinc supplementation can reduce the incidence of infection, oxidative stress, and inflammatory cytokines [23- 25].

Many factors affect the susceptibility of infection in DS sufferers, including immunity, anatomy, and nutrition. Thus, attention is needed for both health workers and caregivers to increase protection, prevent disease, maintain adequate nutrition, and the hygiene of patients. These are expected to improve patient's quality of life and reduce the morbidity and mortality due to infection.

The weaknesses of this study were not examining the number of B lymphocytes and IgG levels in the age group below five years and the adult age group. This study also did not compare between infected and uninfected DS patients. For that, we need further research to prove it. It also needs to prove which immune cell disorders (cellular or humoral) play the most crucial role in causing susceptibility to infection in people with DS.

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Authors' Contribution
The first and second authors prepared and submitted the proposal for the research grant. The first author conducted the research procedures. The first and second authors analyzed and interpreted the results, then developed this manuscript.

Conflict of Interests
All the authors declare that they have no conflicts of interest in publishing this research article.

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3. Conclusion
The conclusion of this study is the number of lymphocyte B cells is lower in DS patients. There is a significant difference in the number of lymphocyte B cells between DS patients compared to controls. The decrease in the number of lymphocyte B cells can be one of the indicators of infection susceptibility in DS. It should be the concern of both health care workers and families of DS sufferers. For this, the infection should be prevented, and immunity should be improved in patients with DS through adequate nutrition and attention to the cleanliness of patients.

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