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RESEARCH ARTICLE

Asthma Exercise Enhanced IL-10 Secretion but Had No Effect on IFN-ɣ in Patients with Persistent Atopic Asthma

Rahmaya Nova Handayani[1,2*], Faisal Yunus[3], Ermita Isfandiary Ibrahim Ilyas[4], Iris Rengganis[5]

1. *Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.*
2. *Bachelor of Nursing Study Program, Harapan Bangsa University, Indonesia.*
3. *Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.*
4. *Department of Medical Physiology, Faculty of Medicine, Universitas Indonesia, Jakarta,Indonesia.*
5. *Departement of Internal Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.*

Corresponding Author:** ***Rahmaya Nova Handayani

Abstract

Background: Asthma is a chronic respiratory disease which is considered as a frequent non-communicable disease that could affect the quality of life. It is widely known that Th1 and Th2 imbalance is one of the underlying pathogenesis of asthma and many approaches had been devised to interfere this process. One of the well-known non-pharmacological approaches in asthma is asthma exercise. Therefore, this study aimed to investigate the role of asthma exercises on the balance of Th1 (IFN gamma) and T-regulator (IL-10). Methods: A pre-post-test group quantitative study was conducted using 39 subjects with persistent atopic asthma. All subjects participated on asthma exercise program that was conducted twice a day, 60 min per session. Atopic status was determined by skin prick test while IFN-Ɣ and IL-10 was determined by ELISA. Results: Median level of IL-10 was increased from 1.6 pg/ml before intervention to 2.2 pg/ml after intervention (p=0.000). However, the exercise program failed to produce any effect on IFN-Ɣ level which only showed a slight median increase from 9.374 pg/ml before intervention to 10.238 pg/ml in the post-test analysis (p=0.07). Conclusion: Asthma exercises significantly increased IL-10 level, but not significantly affect serum IFN-Ɣ in patients with persistent atopic asthma.

Keywords: *Asthma exercise, IL-10, IFN-Ɣ, T-h1/T-h2 balance.*

Introduction

Asthma is a chronic respiratory disease which has considerable potential in reducing the quality of life. World Health Organization (WHO) estimated that the prevalence of asthma at 300 million people worldwide and this number is expected to continue to grow by 200.000 people each year along with a rapidly growing industry. Moreover, more than 80% of deaths from asthma occur in developing countries [1, 2].Based on a survey conducted by the Global Initiative for Asthma (GINA) 2018, it was found that worldwide asthma cases reached 300 million which highlighted its seriousness and predicted to reach 400 million by 2015, with the majority of the cases will occur in developing countries. Asthma is the fifth leading cause of death in the world because its prevalence with an estimated 1: 250 people die from asthma[3, 4]. Asthma is a respiratory disease due to impaired lung function because of bronchial hyperactivity. Bronchial hyperactivity is defined as excessive bronchial constriction caused by direct or indirect stimulation by allergens, chemical irritants, cold air, viral infections, and drugs. The pathogenesis of bronchial hyperactivity is related to the release and production of inflammatory mediators both in the airway epithelial cells and airway smooth muscle cells which are the main pathogenesis of inflammation in asthma [5]. It characterized by infiltration of the airway wall by diverse effector cells such as T-lymphocytes, eosinophils, monocytes/ macrophages, mast cells, and, occasionally,

neutrophils. IL-10 is a pleiotropic cytokine that can exert either immunosuppressive or immunostimulatory effects on a variety of cell types. IL-10 is a potent inhibitor of monocyte/macrophage function, suppressing the production of several pro-inflammatory cytokines.

The pathophysiology of atopic asthma often marked by increased level of IL-4, IL-5, Immunoglobulin (IgE) and eosinophil accompanied by decreased IL-10 level. On the other hand, there is no increase in IgE, eosinophils, and IL-5 in non-atopic asthma albeit there was a relatively small change in IL-10 [6, 8]. Inhalation of antigens activates mast cells and Th2 cells in the airways.

This situation stimulate the production of inflammatory mediators such as histamine, leukotrienes, and cytokines such as IL-5. Impaired balance of Th1 and Th2 will lead to excessive IgE production and results in allergies. These cytokines orchestrate in the recruitment and activation of different effector cells, such as eosinophils and mast cells. These cells along with Th2 cytokines are key players on the development of chronic allergic inflammatory disorders, usually characterized by airway hyperresponsiveness, reversible airway obstruction, and airway inflammation. Accumulating evidence has shown that altering the cytokine-producing profile of Th2 cells by inducing Th1 responses may be protective against Th2-related diseases such as asthma and allergy.

Interferon-gamma (IFN-gamma), the principal Th1 effector cytokine, has shown to be crucial for the resolution of allergic related immunopathologies. Reduced production of this cytokine has been correlated with severe asthma [9, 10].While asthma often treated by pharmacological approaches, several non-pharmacological options can also be considered. One of the emerging non-pharmacological approaches in asthma treatment is asthma exercise. However, lack of study hinders the widespread application of this approaches as well as the clarity of its physiological effect on asthma patients

despite a national standard had been applied to asthma exercise. Therefore, this study aimed to delineate the effect of asthma exercises toward Th1 (IFNgamma) and Th2 (IL-10) balance.

Methods

Pre-post-test groups study using 39 subjects with mild-moderate persistent atopic asthma was conducted to evaluate the study objective. Asthma exercise was led by 3 trained gymnastic instructors from the Purwokerto Community Lung Health Center with several stages of movement namely warming-up, stretching, core exercise a, core exercise b, aerobics-1, -2, -3 and cooling.

Asthma exercise was done in 2 sessions with morning classes began at 6:00 a.m indoor and afternoon classes began at 14:00 a.m outdoors. Asthma exercise was carried out for 60 minutes with an intensity of 70% x (220 - age). The asthma exercise protocol has been registered in the Thai Clinical Trial Registry with the register number is TCTR20190723003.

Skin Prick Test

Skin prick test (SPT) was used to determine the atopic status of the asthma. The allergens used in this test were house dust mite allergens: *Dermatophagoides pteronyssinus*/der p; *Dermatophagoides farinae*/der f; *Blomia tropicalis*/blom t; temoin (negative control); and histamine (positive control). The test was considered to be positive if the wheal diameter is more than 3 mm.

Peripheral Blood Mononuclear Cell (PBMC) isolation

The blood was drawn from every subject and PBMC was subsequently isolated and cultured. 6 ml of blood was homogenized in the heparin falcon and 6 ml of Roswell Park Memorial Institue (RPMI) was added. The RPMI blood mixture was transferred into a ficol tube and the blood was inserted slowly through the walls and then centrifuged at 3000 rpm for 30 minutes.

After the layers were formed, the new buffy coat was transferred into the falcon tube and homogenized with 8 ml stock RPMI. The mixture was centrifuged at 1500 rpmi for 10 minutes to form a supernatant or pellet by adding 2 ml of complete rpmi.

IL-10 Examination

ELISA was used to determine the concentration of IL-10 and IFN-Ɣ in blood sample.

200 µl of sample was used for each well in ELISA plate, in which two rows of the plate wells were reserved for control or standard. The plate then incubated for 2 hours at room temperature, and then washed with a 400 µl washing buffer. 200 µl of IL-10 conjugate was added to each well then incubated for 1 hour at room temperature before second wash. 200 µl of substrate solution was added to each well and incubated for 20 minutes (protected from light). Finally, 50 µl stop solution was added to each well and ELISA reader was used to determine the absorbance of each sample with a wavelength of 450 nm.

Fitness Test 6 Minutes Running Test

All subjects were encouraged to have adequate rest the night before the training test and the training test was conducted at least 2 hours after breakfast. The subjects were informed to take the training test calmly. However, all participants were not allowed to exercise before the training test. For the 6-minute test run, the tracks for walking were marked with track markings. The first marking was made at 15 m and then every three meters. Signs were given at the rotating place of the track.

If the subject stopped, their place was marked with a marker. All of the activities were performed indoors, flat and hard surfaces without obstacles and sufficient lighting. Before the training test, body weight, height, blood pressure, resting pulse, and oxygen saturation were measured by oximetry and a borg scale assessment in the sitting position. The subject started at the marked starting point and walked as much as possible for 6 minutes and stopwatch was used to measure the time. After completing the 6-minute walk test, the stop location was marked.

The distance traveled was measured from the starting place until the end mark. Blood pressure and oxygen saturation were measured again as well as the Borg scale. All of the results were recorded in training test form. The test was stopped if the subjects experienced one of more of the following conditions: oxygen saturation below 80%, chest pain, shortness of breath, leg cramps, diaphoresis, and pale. In addition, if the systolic blood pressure was more than 200 mmHg or less than 60 mmHg, resting the pulse more than 120 times/minute or less than 50 times/minute, the test is stopped.

Research Ethics

Ethics permit has obtained from FKUI ethics committee license number 998 / UN2.F1 / etik / 2017. Permission was also obtained from Margono Soekarjo Hospital in Purwokerto with number 420/2/276 / X / 2017.

Results

According to the baseline characteristics, women has higher proportion compared to men in this study (76.9% vs 23.1%) with most of participants were between 18 to 65 years old. Almost all subjects had normal BMI (94.9%) and fitness level (92.3%) while almost half of the subjects had higher educations (46.2%). Regarding the degree of asthma, most of the subjects were diagnosed to have light persistent asthma (64.1%). The detail of the baseline characteristics can be found in Handayani et.al [11].

Our data shown that the level of IL-10 increased in the majority of subjects (32 subjects) while it decreased in 5 subjects. Two subjects had rather stable level of IL-10 after the intervention. Wilcoxon test was used to analyze the difference between those subjects and the result showed that the median level of IL-10 was significantly increased from 1.6 pg/ml (0.52-5.7 pg/ml) to 2.2 pg/ml (1.4-9.3 pg/ml) with p-value = 0,000 (α = 0.05).

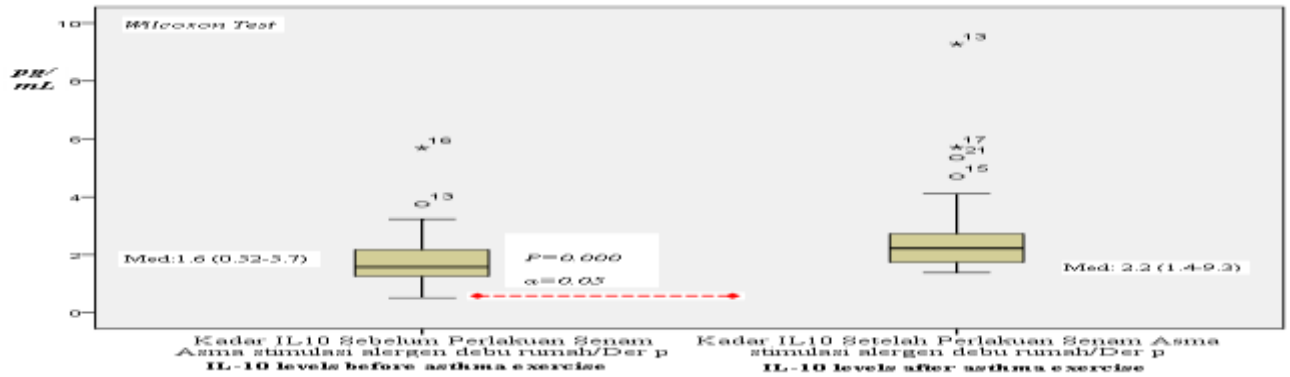

Figure 1: IL-10 levels before and after asthma exercise in Derp p house dust mite stimulation

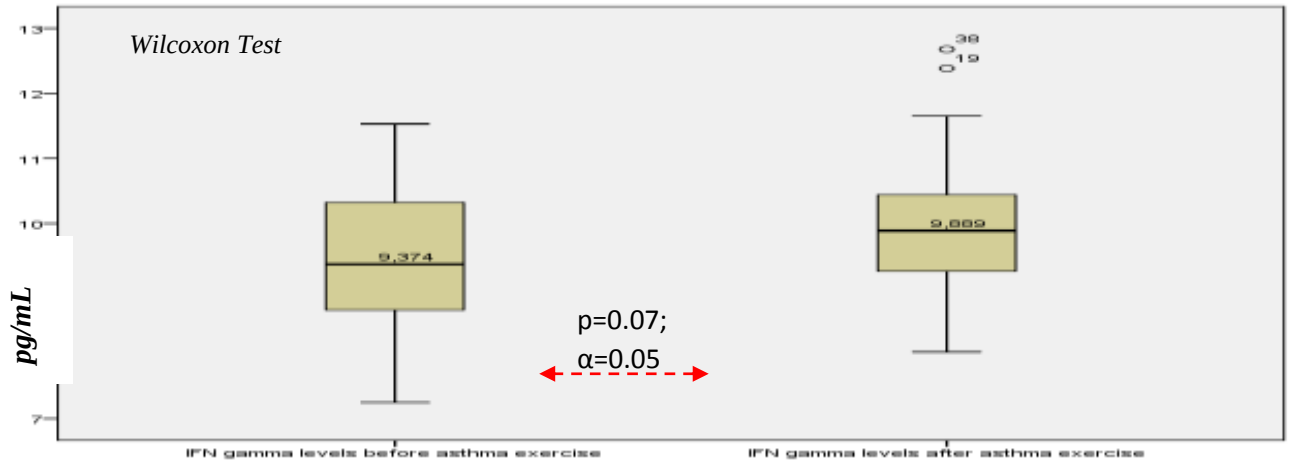

Figure 2: IFN γ levels before and after asthma exercise

In contrast to IL-10 level, IFNγ level among subjects seemed to rather stable. The data showed that 16 subjects had stable level of IFNγ while 23 subjects had elevated level of IFNγ. Only five subjects experienced decreased level of IFNγ. Wilcoxon test showed that the median value of IFNγ level was slightly increased from 9.374 pg/ml (9.234-10.238 pg/ml) before exercise to 9.889 pg/ml (9.274-10,360 pg/ml) after exercise program with p-value = 0.07 (α value =0.05)

Discussion

This study is the first one that showed the effect of asthma exercise toward immunological status in asthmatic patient. The results of this study showed that exercise intervention given 4 times a week for 8 weeks significantly altered the immune profile characterized by increased IL-10 production from PBMC cells taken from subjects who stimulated by allergens to house dust mites. Allergic diseases themselves are affected by environment and genetic factors that could change the balance of Th1 and Th2 immune responses. Ortiz et al showed that genetic factor was indeed plays important role in altering immune response toward allergy.

The allergic response itself can be classified into sensitization phase (antigen exposure) and reactive phase when the level of IgE has increased sufficiently to induce allergic reaction [10]. Type 1 hypersensitivity forms the basis in the pathogenesis of extrinsic asthma that begins with the sensitization of naive T-cells (Th0) that develop into Th1 or Th2 depending on antigenic nature, APC characteristics, cytokine types, and their concentrations. Specifically, Th2 is known to drive anti-body production by secreting IL-4, IL-5, and IL-13.

Their activation is regulated by Treg cells that secrete immunosuppressive IL-10 that has pleiotropic effects (antioxidant, anti-inflammatory, inhibition of cell proliferation, anti-cariogenic and anticoagulant). IL-10 is also produced by $CD4^+$ Th2 cells to regulate Th1 activation. Evidences also emerged that the balance between Th1 and Th2 could be altered through physical activity. Onur and Yalçın showed that exercise had immunomodulatory effect in asthmatic patient and enhances IL-10 level. It is particularly prominent in training with long duration/endurance compared with short-duration exercise. In contrast, short duration intensive training or eccentric contraction exercises tends to produce insignificant cytokine responses. Therefore, cytokine responses are related to various aspects in training including intensity of exercise and psychological stress/burden [12].

In theory, physical exercise with low intensity (40% vo2max) has not been able to stimulate significant change in IL-10 secretion [12]. In the 2010, Lowder et.al showed an increase in T-reg response (IL-10) in mice stimulated by ovalbumin that received moderate-intensity exercise. The authors also stated that changes in cytokines during physical exercise were expected to improve metabolism, tissue repair and response to inflammation [13]. Giron et.al and Benini Et. al stated that sex also play a major role in affecting IL-10 production [14, 15].

As the result, women tend to have greater Th2 (IL-10) response than men. It appears that estrogen and progesterone are the major players that augment IL-10 production in women. The use of oral contraceptives can also affect IL-10 levels in women without physical exercise. In addition, the physiological response to muscle stretch can also trigger cytokine production [12, 15]. Jankord and Jemilo study also confirmed the aforementioned description.

It showed that there was a decrease in IL-6 and an increase in IL-10 in healthy elderly people who did physical exercise regularly. Therefore, it was apparent that physical exercise could modulate inflammatory cytokines response and, hence, immune response [16]. It is also known that IL-6 is synthesized and released into the systemic circulation in response to acute physical exercise. IL-6 contributes the most to the exercise response due to its stimulation by muscle contraction.

An exercise that induces the release of IL-6 depends on the intensity and duration of exercise which affected by changes in calcium balance, glucose level and increased ROS formation that can activate transcription factors in the liver, adipose tissue, and Hypothalamus Pituitary Adrenal (HPA) axis [17]. IL-6 inhibit the development of Treg and contribute to decreased lung function in asthmatic subjects [18]. Based on previous studies, researchers also assumed that one of the factors that also influenced IL-10 level was IL-6, although IL-6 level in this study was not evaluated. In contrast to IL-10, this study showed that exercise had no effect in IFN-ɣ level.

This result is contradicted with Zamani et.al that showed that moderate-intensity physical exercise improved IFN-ɣ level in healthy subjects [18]. In contrast, Kaya showed that physical exercise for 4 weeks did not result in a significant increase in IFNγ in taekwondo athletes [19].An enhanced th2 immune response and the increased level of Th2-specific cytokines such as IL-4, IL-13, and IL-5 contribute to the induction of allergy and asthma. IFN-ɣ, a Th1 cytokine, acts in conjunction with th2 (IL-4, IL-13, and IL-5) in maintaining chronic allergic inflammation [5].However, the mechanisms leading to an enhanced Th2 response are still controversial.

Th2-dominated immune responses may result from immune suppression of T-regulatory cells as well as Th1 cells [8].

Understanding early-life immune mechanisms responsible for atopic diseases, specifically how cytokines of Treg cells act to balance the Th1 and Th2 immune response, continues to be a fruitful area of research. Despite the findings in this study, there are several weaknesses that should be addressed in order to generalize the results of this study.

First, the subject selection did not differentiate between male and female and female was predominant in the subjects in this study which could alter the balance of cytokines that being evaluated (IL-10 and IFN-ɣ). Second, the number of subjects was still considerably small and larger number of subjects might be required to narrow the standard deviation/error. Regardless, the findings highlight the effectiveness of exercise in asthmatic patients and open up a possibility for its combination with pharmacological interventions.

Conclusion

Asthma exercise significantly increases IL-10 level in patients with atopic asthma stimulated by Der p house dust mites but had no effect on IFNγ level. Further studies with controlled subject selection based on sex, age, and family history with higher number of subjects are required to validate the results of this study and to justify its generalization.

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