

Changes of Interleukin-6 (IL-6) and Immunoglobulin G (IgG) in Respiratory Exercise

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Abstract

Background: Skeletal muscle contraction in exercise will synthesize and release Interleukin-6 (IL-6) into the systemic circulation. IL-6 is a pleiotropic, proinflammatory and anti-inflammatory cytokine. IL-6 in exercise can modulate the immune response and metabolism in the liver, adipose tissue, Hypothalamus-hypophyse-adrenal (HPA). **Objectives:** To analyze and prove changes in levels of Interleukin-6 (IL-6) and Immunoglobulin G (IgG) in respiratory art sports of a exercise club. **Methods:** Thirty-four members of the breathing arts sport who met the inclusion criteria, male sex, adult age (≥ 21 years), willing to participate were enrolled in this study. Examination of IL-6 and IgG levels were performed by using Elisa method. The kit for IL-6 used Human IL-6 Immunoassay and IgG level used Total Human IgG Assay **Results:** The t-test results obtained $p = 0.027$ ($p < 0.05$), there was a change between IL-6 pre-test and IL-6 post-test of breathing exercise for three weeks. The t-test results obtained $p = 0.000$ ($p < 0.05$) there was a change between IgG level of pre-test and IgG post-test of breathing arts sports treatment, this change increased significantly. **Conclusion:** There was a change in levels of IL-6 and IgG in the breathing exercise.

Keywords: Adipose tissue, breathing exercise, IgG, IL-6

Introduction

Physical, emotional, and environmental challenges are some of the stressors of human life and this results in a physical response of mild to severe illness ¹. The influence of globalization and the current economic pressures has changed the lifestyle and socio-economic community. This change makes people become stressed and lack of movement and lack of exercise. This leads to an increased risk of metabolic and degenerative diseases such as heart disease, kidney, lung, hypertension, diabetes, obesity, and immune disorders ².

The impact of stress on physical health can be reduced by exercising regularly. Sports health is a physical activity to maintain health and improve body condition. Respiratory exercise in mild hypoxia conditions can stimulate the increase of various cytokines and stimulate more strongly as stress immunologic response compared

to other sports ³. Breathing exercises of Satria Nusantara with special methods are: the breath, movement, and concentration (pray, spiritual, meditation) which is a physical exercise, mental and social exercise ⁴. Satria Nusantara breathing exercise can improve physical fitness and improve body immunity ⁵.

Skeletal muscle is the largest organ in the human body. Skeletal muscle contractions in exercise will synthesize and release myokines or cytokines called Interleukin-6 (IL-6) to the interstitial and systemic circulation. This myocyte or cytokine affects the metabolism, the nervous system, the endocrine system, and the immune system that plays a role in the maintenance of homeostasis ⁶. Contraction of skeletal muscles when a person is exercising can contribute greatly to the levels of the IL-6 present in the circulation.

Increased levels of IL-6 during exercise include levels of other cytokines, such as IL-1 receptor antagonists (IL-1ra), Tumor necrosis factor receptor (TNF-R), and IL-10 ³. The magnitude of elevated levels of IL-6 induced by exercise response depends on the intensity, duration and model of exercise ⁷. The

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synthesis of IL-6 in muscle contraction occurs through mechanisms: increased calcium influx, hepatic glucose availability, and increased formation of Reactive oxygen species (ROS), this will activate transcription factors governing IL-6 synthesis⁷.

Interleukin-6 (IL-6) is a pleiotropic, proinflammatory and anti-inflammatory cytokine. IL-6 during exercise can modulate immune and metabolic responses in the liver, adipose tissue, Hypothalamus-pituitary-adrenal (HPA) and leukocytes. IL-6 plays a role in the differentiation of B lymphocytes into plasma cells that produce immunoglobulins⁷.

Immunoglobulins are glycoproteins that obtained in blood and other body fluids, which contain antibodies and are produced to protect the body against pathogens. Antibodies formed in the primary immune response are generally Immunoglobulin M (IgM), whereas in the secondary immune response is Immunoglobulin G (IgG). IgG is the highest level of immunoglobulin, which is up to 50-80% of all immunoglobulins in the body. Immunoglobulin levels in serum are used to assess the integrity of immune system function⁸.

Satria Nusantara's Respiratory Breathing exercise mechanism to increase body immunity until now has not been thoroughly studied, especially the change of IL-6 and IgG levels in Satria Nusantara's breathing arts sport is still unclear and require further study. The study aimed to determine the changes levels of IL-6 and IgG in Respiratory Exercise.

Method

This study was a laboratory study of quasi-experimental analysis and type with one group pretest-post-test design without the control group (comparison) by measuring levels of Interleukin-6 (IL-6) and Immunoglobulin G (IgG) between before and after treatment breathing sports art Satria Nusantara.

The study was conducted in the sports arts community of Satria Nusantara Surabaya. Laboratory examination of IL-6 and IgG levels were performed by Elisa method in Department of Clinical Pathology, Faculty of Medicine, Dr. Soetomo Teaching Hospital Surabaya. The study was conducted for 5 months, beginning with library search until presentation of research report. Treatment is conducted in August to September 2013.

The sample used was the member of the sport by 34 members of the sport of breathing art, the basic level of hard control, previously had regular practice 2x/week regularly, with the intention did not require the stage of introduction of motion stance first because it was accustomed and have mastered the breath, stance, concentration correctly, and meet the criteria of the sample.

Inclusion criteria are respiratory sports artist Satria Nusantara Surabaya branch, adult age (≥ 21 years), male gender, regular practice 2x/week regularly, and willing to participate in the research. While for the exclusion criteria are members who have coronary heart disease, unregulated diabetes mellitus, suffering from systemic infectious diseases, suffering from immunodeficiency, suffering from malignancy, receiving immunosuppressive and antioxidant therapy. The subjects declared drop-out if not exercising more than 1 time out of 9 exercises during the treatment program to be followed or if the absence of more than 12%.

Working procedure begins with members who have met the inclusion criteria carried out the recording of basic data in the form of the name, age, gender, address. Members who are willing to be subjects of research then sign a consent letter following research and medical action as well as medical action information. Prior to the treatment of Satria Nusantara's breathing art program, blood sampling was performed through the vein mediana kubiti. Blood sampling prior to treatment to obtain baseline values of IL-6 and IgG levels. After that, the research subjects follow the treatment of sports art program Satria Nusantara breathing, for 3 weeks, optimal frequency 3 times a week with duration 90 minutes once the practice. If the last exercise was completed, immediate blood retrieval was performed no more than 1.5 hours after treatment, this is because the half-lives of IL-6 \pm 1.5 hours.

Examination of IL-6 levels was performed using Elisa method. The kit used is Human IL-6 Immunoassay, Quanticine Elisa from R & D Systems. The examination of IgG level was performed by using Elisa method. The kit used is Total Human IgG Assay, Elisa kit from Diagnostic Automation, Inc. USA.

All the collected data was arranged in the data collection sheet in tabular form and processed statistically using SPSS 22.0 (SPSS. Inc. Chicago IL). Univariate

data analysis was used to describe each variable, either independent or dependent variable from case group and control group with frequency distribution table. Furthermore, two-tailed paired t-test two paired t-test.

Results

Characteristics of the sample consist of age, weight, height and body mass index (BMI). The number of samples obtained in this study were 34 samples, with a sample characteristic of 34 men (100%). The mean

age 50.680 years with SD (± 10.2590) and age range between 37-73 years old. Weight has a mean of 66.353 kilograms with standard deviation (SD) (± 11.0189) and the weight range between 43.0-93.0 kilogram. The mean height (mean) in this study was 164.574 centimeters with SD = (± 7.5218) and a height range between 147.5-176.0 centimeters. Body mass index (BMI) has mean of 24.3874 kg/m² with SD = (± 2.8568) and BMI range between 19.53-30.37 kg/m² (Table 1).

Table 1. Research Characteristics

Variables (N=34)	Mean \pm Standard Deviation
Characteristics of Research Subject	
Age(Year)	50.680 \pm 10.2590
Weight (kg)	66.353 \pm 11.0189
Height (cm)	164.574 \pm 7.5218
BMI (kg/m ²)	24.387 \pm 7.5218
Physical Examination Data	
Systole blood pressure (mmHg)	117.940 \pm 6.2910
Diastole (mmHg)	76.470 \pm 4.8510
Pulse frequency(x/minute)	78.590 \pm 1.6720
Frequency of breath (x/minute)	19.060 \pm 1.7220
Body temperature (0C)	36.868 \pm 0.2239
HR max(x/minute)	169.320 \pm 10.2590
75% HR max (x/minute)	127.150 \pm 7.6920
IL-6 levels	
IL-6 pretest	5.098 \pm 1.9528
IL-6 posttest	6.044 \pm 2.0311
IgG levels	
IgG pretest	447.351 \pm 228.5582
IgG posttest	821.559 \pm 177.7086

Note: IL-6: Interleukin-6, igG: Immunoglobulin G

Physical examination is performed before the subjects follow the training program. This physical examination was performed by measuring blood pressure, breath frequency, temperature, and pulse frequency. The results obtained as follows mean systole blood pressure 117.94 mmHg with SD (± 6.2910) and systole blood pressure range between 110-130 mmHg. While diastole blood pressure 76.47 mmHg with SD (± 4.8510) and diastole blood pressure range between 70-80 mmHg. The mean pulse rate was 78.59 x/min with SD (± 1.6720) and a pulse frequency range between 76-80 x/min. The mean breath frequency (sample) in this study was 19.06 x/min with SD = (± 1.7220) and breath frequency range between 76-80 x/min. The mean body temperature of the sample in this study was 36.868 °C with SD (± 0.2239) and the temperature range was between 36.5-37.0 °C (Table 1).

The intensity of the specified workload load is submaximal, determined by the Target heart rate (THR), in a manner calculated with 70% max HR. While maximal HR was calculated with the maximal HR formula = (220-age). The maximum mean frequency of samples was 169.32 x/min with SD (± 10.2590) and maximum HR range between 147-183 x/min. The average frequency of THR or 75% HR was 127.15 x/min with SD (± 7.692) and maximal 75% HR range between 110-137 x/min (Table 1).

The mean IL-6 pre-test level was 5.0918 pg/mL with SD (± 1.95284) and the mean IL-6 level was 6.0444 pg/mL with SD (± 2.0311) (Table 1). The mean rate of pre-test IgG 447.3509 mg/dL with SD (± 228.5582) and mean post-test IgG was 821.5597 mg/dL with SD (± 177.7086) (Table 1).

Table. 2 Two-sided test results (2-tailed)

Variable (N=34)	T	df	Sig.(2-tailed)
IL-6 levels	-2.309	33	0.027
IgG levels	-8.642	33	0.000

Note: $p < 0.05$

Levels of IL-6 have a difference of pre-test and post-test mean is (0.95265). The t-test $H_0: \mu \text{ pre-test} = \mu \text{ post-test}$, gives the value $t = (-2.309)$ with degrees of freedom = $(n-1) = (34-1) = 33$. The SPSS output gives the p-value for two-tailed test = (0.027) (Table 2). Two-tailed t-test results proved that there was a change between IL-6 pre-

test and IL-6 post-test of breathing exercises of Satria Nusantara art for 3 weeks, the change was significantly increased ($p < 0.05$).

While the mean difference of IgG level of pre-test and post-test was (374.2089). From the table Paired sample test also obtained $\bar{d} = (-374.2089)$ and $S_d = (252.4772)$ (Table 2). Two-tailed t-test results proved that there was a change between pre-test IgG and post-test IgG post-breathing exercises of Satria Nusantara respiratory art for 3 weeks, this change was significantly increased ($p < 0.05$).

Discussion

Based on two-tailed paired t-test analysis of parameters of IL-6 and IgG parameters between pre- and post- there is a change so that this result is in accordance with some previous research which states if respiratory sports satria nusantara can increase physical fitness and body immunity ^{5,9}. However, other studies suggest that the study was conducted on male students of the 2nd grade Madrasah Aliyah Mu'alimin Yogyakarta who met the inclusion criteria, the result that respiratory exercise of Satria Nusantara can increase beta-endorphin, IgG and IL-6 levels, while IL-2 and IL-4 did not increase, and cortisol levels decreased ⁹.

Increased levels of IL-6 pre-test and IL-6 post-test treatment of breathing art Satria Nusantara. The resulting increase in IL-6 levels is due to increased IL-6 secretion during exercise physical exercise. IL-6 can increase to 100-fold in physical exercise ⁷. The IL-6 response was sensitive to exercise intensity, indirectly representing the large muscle mass involved in contraction. Exercise that only involves limited muscle mass, was not sufficient for improving IL-6. Exercise involving large muscles resulted in significant increased IL-6. Increased IL-6 during exercise can be inhibited by antioxidant vitamins C and E as well as drugs Indomethacin (class of NSAIDs). This regimen will inhibit NF-kB activity and inhibit elevated levels of IL-6 in response to exercise ¹⁰.

IL-6 might cause some effects in some tissues. IL-6 has catabolic properties, shown by the ability to increase energy expenditure, increase lipolysis, increase fat oxidation, increase endogenous glucose output (by reducing insulin signals in fat and liver), and increase cortisol. The apparent difference between tissues regarding the response to IL-6 may be due to IL-6 signals in different tissues. IL-6 which was released from muscle contraction can stimulate an anti-inflammatory response

reflected increased IL-1ra, IL-10, CRP, and cortisol without elevated pro-inflammatory mediators^{7,11}.

Serum IgG levels in individuals who were given physical exercise for 45 minutes (acute moderate exercise) increased levels of IgG and IgM. This increase was due to extravasation blood proteins, increased lymph flow, noradrenergic sympathetic neural influences on immune responses, or many immunogens inhaled along with respiratory air, whose flow increases during exercise, and the destruction of the mucosal immune response that dries up during exercise. This level decreases after 1.5 hours of exercise and when the exercise is increased with a marathon or run of 45-75 km (ultramarathon) then the second level of the immunoglobulin decreases¹².

Other studies have suggested that runners athletes will decrease cadres of IgM, IgG and IgA after undergoing heavy exercise¹³. However, it was different from other studies suggesting that short rest periods during exercise will not suppress IgA secretion, but increase cortisol levels. Exercise with moderate and regular intensity will provide a good effect for the immune system with the occurrence of increased immunoglobulin. It was because exercise with a heavy intensity will suppress some parameters of the immune system, among others: Ig A, IgG, and IgM¹⁴. Several other studies have also suggested that weight training until exhausted in the morning and evening has a significant effect on the humoral immune system (IgG, IgA, and IgM) and serum cortisol¹⁵.

Exercise sports a pleasant breathing art will nourish the body and also improve body immunity. The existence of abdominal pressing and abdominal pressing during the motion exercise motion of Satria Nusantara, as an effort to guarantee the implementation of breath arrest during the movement. Abdominal pressing does not cause a disturbance of physiology, and in contrast to thoraco-abdominal pressing (manoeuvre valsava) which can cause physiological disturbance and harm because it will raise blood pressure at the beginning of pressing, followed by decreased blood pressure due to retardation of reverse blood flow and venous pressure will increase. In preliminary exercises need to be explained and emphasized the difference between respiratory chest with abdominal breathing in exercise Satria Nusantara^{16,17}.

Abdominal breathing will smooth the blood flow back from the vein in the abdominal area to the heart,

inspiration time (sucking breath) pressure in the abdomen increased and the pressure in the chest cavity decreased. Increased pressure inside the abdominal cavity causes a sort of massage to the organ in the abdominal cavity, a mechanical stimulation of the digestive tract of food, and will improve peristaltic movement. Abdominal breathing also trains the respiratory muscles and abdominal wall muscles^{16,17}.

Conclusion

There were changes in levels of Interleukin-6 (IL-6) and Immunoglobulin G (IgG) in the breathing art of Satria Nusantara. Increased levels are still within the normal range and good in improving body immunity.

Ethical Clearance: This study protocol was approved by ethical clearance Dr. Soetomo Teaching Hospital Surabaya, Indonesia.

Conflict of Interest: The author reports no conflict of interest of this work.

Source of Funding: This study is done with individual funding.

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