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Interleukin-6 as Immune System and Inflammation Biomarker on the Response of Basic Pencak Silat Exercise in Perguruan Pencak Silat Perisai Diri, Bojonegoro

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Abstract

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BACKGROUND: Pencak Silat is a self-defense exercise originated from Indonesia and categorized as a high-intensity exercise.

AIM: This research was intended to identify the acute response of Pencak Silat basic exercise toward interleukin-6 (IL-6) as an immune system biomarker on students of Perguruan Pencak Silat Perisai Diri Tulungrejo, Bojonegoro.

METHODS: A number of 26 students (10 boys and 16 girls) of Perguruan Pencak Silat Tulungrejo, Bojonegoro, were participated in this study. The students did the 2 h Perguruan Pencak Silat Perisai Diri, Bojonegoro, training program with 75–85% intensity. The IL-6 serum was measured using ELISA method.

RESULTS: The result of this study showed that the IL-6 serum level in post-2 (12 h after training) (6.2981 pg/mL) was higher compared with the IL-6 serum level in post-1 (shortly after training) (6.11981 pg/mL) and before training (4.5146 pg/mL). The result also showed that there was a significant difference of IL-6 levels between pre-training and after training.

CONCLUSION: This study concluded that the basic exercise performed by the new students of Perguruan Pencak Silat Perisai Diri increased IL-6 serum level.

Introduction

Previous theory showed that there are changes in body's immune system due to acute exercise that occurs between 3 until 72 h after exercise, although it depends on the parameters measured [1]. The previous study also showed that there was a risk in diseases transmission after doing high-intensity physical activity [2]. This is due to the depression of body immune system that occurs after high-intensity exercise as explained in J-curve theory, in which the moderate-intensity level of physical activity could increase body immune system, while, in contrast, prolonged high-intensity level of physical activity could alter body immune function [3]. Exhausting physical activity could lead to exaggerated inflammatory reaction that causes immunity suppression, therefore causing clinical consequence that slowdown the healing and recovery of injury and increasing disease or infection risk [4].

Inflammation is a complex event that involves lots type of cells and molecules that are called pro-inflammatory cytokines. These cytokines are the earliest product as a response toward stress and they have important role as an innate and adaptive immune system. They have function as signal between cells that control local and systemic response. Cytokines can be induced and divided into two different families, the pro-inflammatory cytokines (interleukin [IL]-1 β , IL-2, IL-4, IL-6, IL-8, and tumor necrosis factor-alpha [TNF- α]) that have function to increase systemic inflammation and inflammatory cytokines (IL-5, IL-6, and IL-10) that have protective and reducing character toward inflammation. Exaggerated inflammation may cause harm and excessive production of IL-1 β , while TNF- α may cause pathological problems [5].

Cytokines is a small protein (<40 kDa) secreted and produced by almost all cells in the body to control and influence immune response. Releasing pro-inflammatory cytokines will trigger the activation

and production of immune cells and release further cytokines. Therefore, the “cytokines storm” term is explaining inflammation as the proses of sudden cytokines release to increase inflammatory process. IL-6 is rapidly produced and acted as temporary response toward infection and tissue injury and has contribution in body’s immune system by stimulating acute-phase response, hematopoiesis, and immune reaction [6].

A previous study reported that there were increments in IL-6 plasma concentration as a response toward physical exercise performed by an individual. Exercise and strenuous exercise will induce the level of several pro- and anti-inflammation cytokines inhibit natural cytokines and chemokines. IL-6 level will increase 100-fold after running marathon race. Higher increment of IL-6 level will be produced as the response toward exercise compared with the other cytokines type. IL-6 is produced locally in skeletal muscle as a response toward exercise, and it is known that IL-6 induces the output of hepatic glucose and inducing lipolysis [7]. Strenuous endurance exercise will cause leukocytosis that occurs due to neutrophilia in circulation, damaging muscle and internal organs, and suppressing immunity. To determine the cause of this phenomenon, attention should be focused on cytokines that are released to the circulation system. A lot of research showed consistent finding that IL-1ra, IL-6, IL-8, and IL-10 have significant increase in prolonged endurance exercise that takes place in longer hours, such as marathon and triathlon [8]. High Mobility Group Box 1 (HMGB 1) is pro-inflammatory cytokines that have important role in sterilized inflammation, infection, until sepsis. Intracellular response will be activated by the binding between HMGB1 and the surface of immune cell that aims to control the function of immunity cells, including chemotaxis and modulation of immune system [9]. There will be increase in systemic HMGB1 after doing aerobic exercise with high intensity, treadmill, marathon run, and 5 min step test in hypoxic room [10].

Endurance exercise is also applied in Pencak Silat. Pencak Silat is a self-defense exercise originated from Indonesia. Basic moves in Pencak Silat consist of punching, kicking, step patterns, defense, and cutting style (guntingan). Like the other physical exercise, Pencak Silat also implements principal exercises that aim to improve physical condition. In Pencak Silat course, new students have to undergo basic exercise, the first exercise from them to adapt before they will be given with the next level of exercise. Basic exercise in Perguruan Pencak Silat Tulungrejo, Bojonegoro, consists of warming up, core exercise, and cooling down, which last for 90–120 min per exercise session. The basic exercise activity of Perisai Diri is dominated by eccentric move such as kicking and punching, also jumping and running. However, the response of immune system toward basic exercise in Perguruan Pencak Silat Perisai Diri has not been thoroughly studied. Therefore,

this research was intended to identify the acute response of Pencak Silat basic exercise toward IL-6 as immune system biomarker in students of Perguruan Pencak Silat Perisai Diri Tulungrejo, Bojonegoro.

Materials and Methods

A number of 26 students (10 boys and 16 girls) of Perguruan Pencak Silat Tulungrejo, Bojonegoro, aged 17 years old, body weight 61 kg, and in healthy condition were participated in this study. This study protocol was already approved by the local ethical committee Komite Etik Penelitian Kesehatan Masyarakat, Universitas Airlangga and all participants had been given information on the risk and the objective of this study before they filled the informed consent. Pencak Silat exercise was done in Perguruan Pencak Silat Tulungrejo, Bojonegoro, East Java, Indonesia. The exercise was using basic exercise method from the Perguruan Perisai Diri with 120 min duration, consisting of warming up, core exercise, and cooling down.

Blood samples were taken from intercutibul blood vessel before (pre) the basic exercise. After that, blood samples were taken shortly after (post-1) and 12 h after the exercise (post-2). The study subjects had not been doing exercise activities in the previous 2 days. The subjects were also allowed to consume any food they wanted, but prohibited from taking supplement and drugs 1 week before the intervention and data collection of data. Blood sample was taken from cubitus vein as much as 5 cc then added with EDTA. Centrifugation process was held in 15 min and in 3000 rpm, then, the blood plasma was saved in a freezer in 20°C temperature. The 100 µL of plasma before (pre), shortly after (post-1), and 12 h after intervention (post-2) were used to count the IL-6 level. To check the IL-6 level, we used Kit Human IL-6 using double antibody sandwich ELISA method.

The collected data were processed manually and digitally to change the data to information. Data analysis used in this study was univariable analysis to portray the subject characteristic. The normality test is used was the Kolmogorov–Smirnov test. Bivariate analysis was also used to compare the difference between IL-6 level before (pre), shortly after (post-1), and 12 h after intervention (post-2). Mann–Whitney U-test was used if the data were not normally distributed and independent-t test was used if the data were normally distributed. This study was conducted on September 2020 until January 2021 in Public Health Faculty, Universitas Airlangga, Surabaya, Indonesia.

Results and Discussion

Respondents' characteristics

A number of 26 students, consisting of 10 boys and 16 girls, with mean age 16 years old (SD \pm 0.9) were students of Perguruan Pencak Silat Tulungrejo, Bojonegoro, and willing to become respondents and participate in this study. Other characteristics of the respondents are shown in Table 1.

Table 1: Respondents' characteristics

Variable	n	Minimum	Maximum	Mean	SD
Age	26	16.00	19.00	26.7308	0.91903
Weight	26	43.6	77.40	60.4000	9.53138
Height	26	147.00	177.00	162.077	7.47138
Valid n	26				

Normality and homogeneity test

Before doing the statistical analysis of IL-6 serum level, data distribution normality test was done for the data of IL-6 level on pre (before), post-1 (shortly after), and post-2 (12 h) after intervention. These variables were tested using Kolmogorov–Smirnov normality test. The result of the statistical test showed that pre-intervention IL-6 serum level had $p = 0.200$ with the Z-score of 0.130, while the p -value and Z-score of post-1 and post-2 were 0.200 and 0.119, respectively (Table 2). From the result, it showed that the data were normally distributed because the p -value was bigger higher than $\alpha = 0.05$.

Table 2: Data distribution of IL-6 serum level

IL-6 serum level	n	Z	p
Pre	26	0.130	0.200
Post-1	26	0.119	0.200
Post-2	26	0.119	0.200

IL-6: Interleukin-6, *one-sample Kolmogorov–Smirnov test.

According to the homogeneity test using F-Levene test, the p -value of the three variables was $p \leq 0.001$. Therefore, we can conclude that the variety of the variables was different with degree of error 5% and these variables were heterogeneous (Table 3).

Table 3: Homogeneity of IL-6 serum level between pre, post-1, and post-2

IL-6 serum level	n	Mean (pg/mL)	SB	Variation coefficient (%)	p
Pre	26	4.5146	0.211161	4.68	≤ 0.001
Post-1	26	6.11981	0.64657	10.57	
Post-2	26	6.2981	0.64657	10.57	

IL-6: Interleukin-6.

The IL-6 serum level in post-2 (6.2981 pg/mL) was higher compared with the IL-6 serum level in post-1 (6.11981 pg/mL) and pre (4.5146 pg/mL). The result also showed that there was a significant difference between the variables using the 95% confident intervals (Table 3).

Comparative test using t-test

The result from analysis using t-test with 95% confident interval showed that there was a difference in

IL-6 serum level between pre- and post-1. IL-6 serum level of pre- and post-2 also showed similar result (Table 4).

Table 4: Result of t-test

Parameters	Paired differences				t	df	Sig. (two tailed)
	Mean	Std. deviation	Std. error mean	95% CI Lower Upper			
Pair 1	-1.683	0.734	0.144	-1.979 -1.388	-11.717	25	<0.001
Pre-post-1							
Pair 2	-1.783	0.734	0.144	-2.079 -1.488	-12.413	25	<0.001
Pre-post-2							

The result of comparative test using t-test between pre- and post-1 had significant result ($p \leq 0.001$). This showed that there was a significant difference between IL-6 serum level in pre- and post-1. Along with the result, comparative test of pre- and post-2 also showed similar result that there was significant difference between IL-6 serum level in pre- and post-2 ($p \leq 0.001$).

Perguruan Pencak Silat Perisai Diri Tulungrejo located in Bojonegoro is one of the self-defense courses in Indonesia. This course had exercise program aimed to increase the physical ability and condition. One of the exercise programs given to the student is the basic exercise program. This program is given so that the student will be ready and able to adapt before receiving the next level exercise. The moves in the basic exercise are varied, consisting of sprint, jogging, jumping, punching, kicking, and sweeping (Sapuan). This basic exercise is categorized as high-intensity interval training (HIIT). Exercise in this category could cause suppression in many aspects of the body immunity function, such as the increase of neutrophil, lymphocyte proliferation, and the presentation of monocyte antigen. This study aims to identify the acute response of Pencak Silat Perisai Diri basic exercise toward IL-6 as the biomarker of immune system on among the students of Perguruan Pencak Silat Perisai Diri Tulungrejo, Bojonegoro.

The results showed that there was difference in IL-6 serum level before the intervention, shortly after the intervention, and 12 h after the intervention. This showed that the intervention (the basic exercise of Pencak Silat) could increase IL-6 serum 1.60521 pg/mL (35.56%) shortly after the intervention and 1.7835 (39.51%) after 12 h of the intervention. Prolonged strenuous exercise could cause temporary depression in many aspects of immune function that will occur 3–24 h after the exercise. Immune system dysfunction may occur after exercising (post-exercise), especially in prolonged exercise (1.5 h) with moderate to high intensity (55–75% VO_2 Max). The findings in this study by Gleesom [3] are consistent with our study because the exercise performed was a HIIT with long duration of approximately 120 min. High-intensity exercise could induce an increase in several pro- and anti-inflammatory cytokines level, natural cytokine inhibitor, and chemokines. As a response to exercise, IL-6 is produced locally in the skeletal muscle in higher level than other cytokines, and this induces hepatic glucose output as well as lipolysis [7].

The IL-6 is a 184 amino acid protein cytokine produced by many types of cells and expressed in cellular stress such as inflammation, infection, injury site, and cancer [11], [12]. IL-6 is also produced at the site of inflammation and functions as a clinical or biological acute-phase response such as the production of acute-phase protein. IL-6 has stimulation effect on B and T cells, and supports chronic inflammation response [13]. This cytokine is also produced quickly and temporarily as a response toward infection and tissue injury has contribution to body's immunity by giving stimulation to acute-phase response, hematopoiesis, and immune reaction. Although its expression is tightly controlled by the transcription and post-transcription mechanism, unregulated synthesis of IL-6 may cause pathological effect in chronic inflammation and autoimmunity [6]. IL-6 is operated to control how far the response of tissue inflammation. In chronic disease, immune stressor, such chronic intracellular infection, and tumor, the IL-6 function is not only to induce acute phase reaction but also to bring up cellular immunity response toward damaged cells and mucosa humoral response that leads to reoccurrence of infection [13]. Plasma IL-6 will increase when someone do heavy activity [7]. The increment of IL-6 is also caused by sepsis [14] and when trauma occurs [15].

The cause of changes in immune system due to exercise is multifactorial. One of the causes is neuroendocrinological factor such as adrenalin (epinephrine), noradrenalin (norepinephrine), growth hormone, and cortisol [16]. The concentration of these hormones is elevated during exercise and get back to normal soon after the exercise. This process also gives effect to lymphocyte and neutrophil during the recovery phase. The previous study using infused hormone showed that the production of hormone was inhibited by epidural blockage in relation with physical stress that provided contribution to action mechanism [17]. Adrenalin and noradrenalin are in charge in acute exercise effect on lymphocyte change, including functional activity of NK-cells and T-cells. The elevated growth hormone and catecholamine become the mediator of acute effects on neutrophil, while cortisol gives its effects at least 2 h. Therefore, it helps to maintain the lymphopenia only after long duration exercise [18]. Other studies also showed that epinephrine become the mediator effect of exercise on lymphocyte concentration. Therefore, intravenous epinephrine in 1 h causes lymphocytosis followed with lymphopenia, which is similar to the effects of exercising [19].

Elevated IL-6 secretion inside the body due to the response toward strenuous exercise is caused by elevated of cytokines inhibitor because of antagonize receptor such as IL-1 (IL-1ra) and anti-inflammatory cytokine IL-10 [20]. The quick elevation of IL-6 secretion that is circulating during exercise is mediated by the elevated regulation of transcription and contraction from the skeletal muscle [21]. IL-6 is the only myokine known that can be released from the skeletal muscle

to the circulation in the concentration needed [22]. IL-6 is also regulated in adipose tissue and released as the response to exercise [10], [23]. IL-6 secreted from adipose tissue also contributes in systemic elevation of IL-6 in exercise [24].

During recovery after exercise, microstructure damage occurs due to microtrauma in contractile element and the connective tissue shows infiltration of immune cells and cytokines secretion, including IL-6, similar to the inflammatory response toward tissue injury or infection. This effect is mostly delayed or as the metabolic effect of IL-6 during exercise, especially in recovery. The peak circulation concentration from IL-6 released from muscle occurs shortly after acute exercise. Meanwhile, after the peak, the level of IL-6 produced by immune cells may be lower or still high for a long duration to recover the damaged tissue [25].

Conclusions

The basic exercise performed by new students of Perguruan Pencak Silat Perisai Diri increased IL-6 serum level. This was because the characteristics of the basic exercise in this course were long and strenuous that could cause muscle damage and stimulate the release of more IL-6 to minimize the risk to the body.

Ethical Approval

This study protocol is already approved by the ethical committee of Public Health Faculty of Universitas Airlangga, Indonesia, in 2020.

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