Differences of Leukocyte and Neutrophils to Lymphocytes Ratio on Maximum Training

Arif Fadli Muchlis[1*], Afriwardi[2], and Efrida[3]

[1,2,3]*Faculty of Sports Sciences, Universitas Negeri Padang, Padang, Indonesia*
**Corresponding author. Email: arif_fmuchlis@fik.unp.ac.id*

ABSTRACT
Physical exercise is a stressor to the immune system. Leukocytes and the neutrophils toward lymphocytes ratio (NLR) can be used to determine the status of immunity. This study aims to see the difference in the number of leukocytes, neutrophils, lymphocytes, and NLR on maximal physical exercise. This research was a laboratory experimental study by using the post test-only control group design to 10 adult males of white mice (Mus musculus) divided into two groups, five mice for the treatment group and five mice for control group. The treatment group was given maximal physical exercise through swimming until visible signs of fatigue were seen, and control group only kept immersed in the water for 20 minutes. The number of leukocytes was assessed by using Improved Neubauer chamber cell counting and NLR was calculated by comparing the number of neutrophils to lymphocytes. The result and statistical analysis using unpaired t-test showed significant differences were found in the leukocyte count (p = 0,000), while NLR was not found its significant difference (p = 0.464). In comclusion, it was found that the number of leukocytes was higher on the treatment group whereas the NLR was not significantly found difference.

Keywords: *Athlete, cellular component, immune system, stressor*

1. INTRODUCTION

Athletes often undergo strenuous physical training both during the training period and during the competition. This situation increases the risk of illness that results in a decrease of physical performance, led to the interruption of the planned training program, and lost the opportunity to take part in important sports competitions that were taking place [1]. [2] Physical exercise has the ability to influence the strength of protection and the body's vulnerability to infection [3]. Physical exercise is a stressor for homeostasis. This is due to an increase in energy requirements during physical exercise. So an attempt was made to maintain homeostasis through a stress response mechanism as a physiological response to stressors. Stress is responded through a stress system that involves the neuroendocrine system that forms the axis of hypothalamic, the pituitary with the adrenal cortex (HPA axis), and the sympathetic nervous system axis with the adrenal medulla [4]. The stress system will produce hormones as the stress response due to physical exercise. The stress hormones produced also result in suppression of the immune system, including cellular components consisting of leukocytes. Suppressing the immune system due to stress will increase the risk of illness [5]. Neutrophils and lymphocytes are types of leukocytes that play a role in natural and adaptive immune responses. [6]. Measurement of the number of leukocytes and the neutrophils to lymphocytes ratio (NLR) illustrates the response to stress and explains the balance of natural and adaptive immune responses [7,8]

Previous research shows leukocytosis, neutrophilia and lymphocytosis occur in various forms of physical exercise [9]. In other studies, it was found lymphopenia occurred after physical exercise [10] Assessment of leukocytes can be a diagnostic reference for assessing the effect of physical exercise on athlete's health because assessing immunity status in athletes is an important part in preparing for a competition [11].

This study aims to determine the differences in cellular components of the immune system that occur after maximal physical exercise through measurement of the number of leukocytes, neutrophils, lymphocytes and NLR in adult males of white mice (*Mus musculus*) which were randomly divided into treatment groups compared to the control group.

2. METHODS

This research was carried out for five weeks in the Immunology Laboratory of the Faculty of Pharmacy of Andalas University and the examination of blood samples was carried out at the Clinical Pathology Laboratory of the Faculty of Medicine, Andalas

University. This research was a laboratory experimental study with the Post-test only control Group design to determine the average difference between the treatment group and the control group.

The data obtained were processed by computer using statistical applications. The mean differences between the two groups was analyzed using an unpaired t- test. The mean difference was significant if $p < 0.05$. The values obtained was presented in tabular form by showing the mean, standard deviation (SD), t and p values.

3. RESULTS

The population in this study was white mice (*Mus musculus*), and the samples used were 10 adult male mice that met the inclusion criteria, in a healthy condition, 8-10 weeks of age and 30-35 gr body weight. The sample was randomly divided into 2 groups: the treatment and control groups and each group consisted of 5 mice.

Before the experiment was conducted, all mice underwent acclimation for 7 days to adjust to the environmental conditions of the experiment. Mice were kept in separated cages of the same shape and size. Each cage was filled with 5 mice. The conditions of temperature, humidity and lighting were naturally obtained. Feeding mice was obtained from standard food and drinks *ad libitum*.

The treatment was given for 7 days for the treatment group mice through swimming exercises for 10 minutes on days 1, 3, and 5, and through maximum swimming until fatiguness signs was shown on the 7th day while the control group mice only soaked in water on the 7th day for 20 minutes.

Blood samples were obtained 2 hours after the trial through the jugular vein, then all the mice were sacrificed. Blood samples taken about 0.5 ml were collected into a tube (GP *micro tube* 0,5 ml EDTA.K3). After the blood sample was obtained, the number of leukocytes was calculated using *Improved Neubauer chamber cell counting*, and making smear preparations with Giemsa staining. Leukocyte count test was done to determine the number of neutrophils and lymphocytes and calculation of the neutrophils to lymphocytes ratio (NLR).

Statistical analysis obtained from the experimental data can be seen in the following tables:

Table 1. Mean value of leukocyte of mice in the treatment and control groups

Trial Groups	Mean ± SD(x 10^3/µl)	T	p
Treatment (n=5)	10,01±1,31	7,164	0,000
Control (n=5)	4,24±1,24		

Table 1 shows that there were significant differences in the number of leukocytes in the treatment and control groups ($p < 0.05$).

Table 2. Mean value of neutrophil of mice in the treatment and control groups

Trial Groups	Mean ± SD(x 10^3/µl)	t	p
Treatment(n=5)	3,91±1,67	3,291	0,023
Control (n=5)	1,32±0,53		

Tabel 2 shows that there were significant differences in the number of neutrophil in the treatment and control groups ($p < 0.05$).

Table 3. Mean value of lymphocyte of mice in the treatment and control groups

Trial Groups	Mean ± SD(x 10^3/µl)	t	p
Treatment (n=5)	6,10±1,82	3,338	0,010
Control (n=5)	2,92±1,11		

Table 3 shows that the number of lymphocytes in the treatment group and the control group there were significant differences ($p < 0.05$).

Table 4. Mean value of neutrophils to lymphocytes ratio of mice in the treatment and control groups

Trial Groups	Mean ± SD	t	p
Treatment (n=5)	0,76±0,59	0,769	0,464
Control (n=5)	0,55±0,42		

Table 4 shows there was no significant difference on the value of the neutrophils to lymphocytes ratio (NLR) in the treatment group and the control group ($p > 0.05$).

4. DISCUSSION

The number of leukocytes on the maximum physical exercise

This study shows the number of leukocytes in the treatment group is higher than the control group. Leukocytosis obtained in this study is in line with the research conducted by Sand *et al*., towards 800 adult men and women divided into 2 groups of 800 adults men and women divided into 2 groups [9]. Leukocytosis in physical exercise is mainly caused by mobilization of neutrophils and lymphocytes. In the initial stages of physical exercise leukocyte mobilization was caused by increasing blood flows (shear stress), and the effects of catecholamines which reduce the expression of leukocyte adhesion molecules in endothelium [12]. Catecholamines are hormones produced by the axis of sympathetic nervous system with the adrenal medulla which the stress system is involved in the process of energy metabolismdan mechanism of muscle contraction during physical exercise [4].

The number of neutrophils in the maximum physical exercise

This study shows the number of neutrophils in the treatment is higher than in the control group.Neutrophilia is also found in studies conducted by Sand [9] and in a study of after participating in 2 matches in 1 day with intervals of rest for 4 hours between the matches [13].

Neutrophils are one of the first lines of the natural immune system. Neutrophils have various abilities to destroy various types of pathogenic forms through inflammatory processes in tissues [6]. Neutrophils are a relatively dominant component of leukocytes which increases in number when physical exercise is performed. Neutrophils that occur during physical exercise are caused by neutrophil margination of the vascular. In the beginning exercise, the demargination was caused by *shear stress*, catecholamines and cortisol [12]. Cortisol also results in the process of slowing down apoptosis in neutrophils [14].

Catecholamines and cortisol as the final products of stress systems play a role in energy metabolism including through the process of gluconeogenesis and lipolysis. Besides that catecholamines have a role to improve the performance of the cardiovascular system and the mechanism of muscle contraction during physical exercise.[4] Margination of neutrophils in the vascular due to the influence of cortisol will result in a decrease of the ability of neutrophil migration into the inflamed tissue [15].

The number of lymphocytes on maximal physical exercise

This study shows that the number of lymphocytes in the treatment is higher than in the control group. Lymphocytosis is also found in studies conducted by Sand[9] and on 8 male tennis players who had done routine training and had finished 10 sets of tennis practice protocols [10].

Lymphocytosis in physical exercise is caused by *shear stress*, catecholamines and cortisol.[12] In contrast to neutrophilia, lymphocytosis in physical exercise is biphasic. Increasing lymphocytes occur at the beginning of physical exercise followed by a decrease in the number of lymphocytes in the early stages of the recovery phase after physical exercise [11]. Decreasing the number of lymphocytes is influenced by the process of redistribution of lymphocyte cells in various organs as a reservoir of lymphocytes and the apoptotic process that is triggered due to the increase of cortisol [14]. Lymphocytes is a component of adaptive immunity which has a special role in specific pathogens through antibody production and the role of memory cells [6].

Neutrophils to lymphocytes ratio in maximum physical exercise

This study shows there is no difference in the neutrophils to lymphocytes ratio(NLR) between the treatment group and the control group. Thi insignificant differences were also found in the group of taekwondo athletes after attending the training for 10 weeks. This situation was found in the taekwondo athlete group with a relatively high NLR value before treatment with an exercise program.[16]An increase in NLR values in athletes is related to fatigue and stress [17].

Every individual has a different response to stress. This situation is caused by differences in perception, adaptation and coping mechanism to stress [18]. In this study, all experimental animals had carried out the acclimatization and adaptation process to the environmentand experimental treatment. The difference in stress response in experimental animals can be influenced by genetic factors, strain, activation of stress hormone receptors and adaptation processes [18].

5. CONCLUSIONS

The number of leukocytes, neutrophils and lymphocytes is higher in the treatment group and there are no differences in NLR values between treatment and control groups.

ACKNOWLEDGMENTS

Thank you to the Head and staff of the Immunology Laboratory, Faculty of Pharmacy and Clinical Pathology, Faculty of Medicine, Andalas University, who helped to carry out this research.

REFERENCES

1. Schwellnus M, Soligard T, Alonso JM, Bahr R, Clarsen B, Dijkstra HP *et al*, How Much is Too Much? (Part 2) International Olympic Committee Consensus Statement on Load in Sport and Risk of Illness. Br J Sports Med. 2016; 50: 1043-1052.
2. Gleeson, M. How Common are Illnesses Amongst Athletes? (Diunduh 3 September 2016). Tersedia dari: URL: HYPERLINK: http://www.mysportscience.com/singlepost/2016/08/19/How-common-are-illnesses-amongst-athletes
3. Schwellnus MP, Jeans A, Motaung S, Swart J. Exercise and Infections. In: Schwellnus MP, editor. Olympic Textbook of Medicine in Sport. Chichester: International Olympic Committee Wiley-Blackwell; 2008. p. 344-364.
4. Fragala MS; Kraemer WJ; Denegar CR; Maresh CM; Mastro AM; Volek JS. Neuroendocrine-Immune Interactions and Responses to Exercise. Sports Med. 2011; 41(8): 621-639.

5. Glaser R, Kiecolt-Glaser J. Stress-Induced Immune Dysfunction: Implications for Health. Nat. Rev. Immunol. 2005; 5: 243-251.
6. Abbas AK, Lichtman AH, Pillai S. Cellular and Molecular Immunology. 7th ed. Philadelphia: Elsevier Saunders; 2012.
7. Davis A, Maney D, Maerz J. The Use of Leukocyte Profiles to Measure Stress in Vertebrates: a Review for Ecologists. Functional Ecology. 2008; 22: 760-772.
8. Azab B, Camacho-Rivera M, Taioli E. Average Values and Racial Differences of Neutrophil Lymphocyte Ratio among a Nationally Representative Sample of United States Subjects. PLoS ONE. 2014; 9(11), p. e112361.doi:10.1371/journal.pone.0112361.
9. Sand KL, Flatebo T, Andersen MB, Maghazachi AA. Effects of exercise on leukocytosis and blood hemostasis in 800 healthy young females and males. World J Exp Med. 2013;3(1): 11-20.
10. Schafer M, Kell H, Navalta J, Tibana R, Lyons S, Arnett S. Effects of a Simulated Tennis Match on Lymphocyte Subset Measurements. Research Quarterly for Exercise and Sport. 2014; 85: 90-96.
11. Kakanis MW; Peake J; Brenu EW; Simmonds M; Gray B; Hooper SL *et al*. The Open Window of Susceptibility to Infection After Acute Exercise in Healthy Young Male Elite Athletes. Exerc. Immunol. Rev. 2010; 16: 119-137.
12. Walsh NP, Gleeson M, Shephard RJ, Gleeson M, Woods JA, Bishop NC *et al*. Position Statement Part one: Immune Function and Exercise. Exercise Immunology Review. 2011; 17: 6-63.
13. Takahashi I, Umeda T, Mashiko T, Chinda D, Oyama T, Sugawara K, Nakaji S. Effects of Rugby Sevens Matches on Human Neutrophil-Related Non-Specific Immunity. Br J Sports Med. 2007; 4: 13-18.
14. Krüger K, Mooren FC. Exercise-induced leukocyte apoptosis. Exerc. Immunol. Rev. 2014; 20: 117-134.
15. Coutinho AE, Chapman KE. The Anti-inflammatory and Immunosuppressive Effects of Glucocorticoids,Recent Developments and Mechanistic Insights. Molecular and Cellular Endocrinology. 2011; 335: 2-13.
16. Chen CY, Liao YH, Chou CC, Sung YC, Tsai SC. Initial Systemic Inflammatory State Appear to Pertubs Exercise training Adaptations in Elite Taekwondo Athletes. PloS ONE12(4):e0176140.https://doi.org/10.1371/journal.pone.0176140.
17. Matsuo K, Kubota M, Sasaki H, Toyooka J, Nagatomi R. The Association of the Blood Lymphocytes to Neutrophils Ratio with Overtraining in Endurance Athletes. New Studies in Athletics. 2009;4(24):23-29.
18. Dhabhar FS. Effects of Stress on Immune Function: Implications for Immunoprotection and Immunopathology. In: Contrada RJ, Baum A eds. The Handbook of Stress Science.New York: Springer Publishing Company. 2011.al fitness. Sports are some of the basic needs in human life because they can improve the fitness needed for doing everyday tasks. Sports activities aimed at health, pleasure and sports achievement.