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Original Research

Effect of Intermittent Fasting on Decreased Interleukin-6 Genes mRNA Expression in Mice (Mus Musculus)

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Abstract

Nowadays, the development of various degenerative diseases is very rapid. The rapid development of the disease has encouraged the wider community to understand the impact. Intermittent fasting can help prevent the emergence of cancer (one of the degenerative diseases) because during fasting the body's cells are in protective mode so as to protect the body from disease. Intermittent fasting reduces the body's inflammatory status by inhibiting the expression of proinflammatory cytokines, especially interleukin-6 (IL-6).

This study aims to examine the effect of intermittent fasting on IL-6 gene mRNA expression. This study is an experiment with Pretest-Posttest with Control Group design to examine the effect of intermittent fasting on the of IL-6 genes mRNA expression. The subjects of this study were 10 mice divided into 2 groups: the intermittent fasting group and the control group. The research was carried out at the Laboratory of Molecular Biology and Immunology Department, Faculty of Medicine, Universitas Hasanuddin, Makassar, as a place for the maintenance and care of experimental animals as well as examination of IL-6 genes mRNA expression. The study was conducted for 30 days.

The results showed a decrease in the average value of IL-6 mRNA expression of subjects before and after intermittent fasting with the value of $\rho = 0.003$ ($\rho < 0.05$) showed that there is a significant effect between intermittent fasting on the decrease in IL-6 genes mRNA expression.

In conclusion, there is a decline in the IL-6 genes mRNA expression after intermittent fasting.

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1. Introduction

Nowadays, the development of various degenerative diseases is very rapid. The rapid development of the disease has encouraged the wider community to understand the impact. Indonesia is faced with the problem of double burden in the health sector, that is, the problem of infectious diseases that have not been completely dealt with, the new problems arise that is the degenerative diseases requiring more expensive health costs. The results of the Basic Health Research 2018 showed the prevalence of degenerative diseases had increased as the prevalence of cancer rose from 1.4 percent (2013) to 1,8 percent in 2018.[1] Degenerative disease is a medical term to describe a disease arises as a result of the process of deterioration of the body's cell functions from normal conditions to be worse or the level of cell activity in the body is decreased. The body experiences deficiency in enzyme and hormone production, immunodeficiency, lipid peroxide, cell damage (DNA) and blood vessels. Degenerative diseases are chronic diseases affecting the quality of life and productivity of people.[2–6] One method to restore body cell function and enhance immunity is by intermittent fasting.[7]

Intermittent fasting is recommended as a medical treatment for a variety of conditions, including weight control, resting digestion, improving blood lipid profile, and treating degenerative diseases[8]. Intermittent fasting helps prevent the emergence of cancer (one of the degenerative diseases) because during fasting the body's cells are in protective mode so as to protect the body from disease. In addition, cancer cells will have difficulty developing because of their food source, glucose is not found in the bloodstream when intermittent fasting[9].

Several experimental studies have proven the health benefits of intermittent fasting by affecting biochemical and physiological functions, increasing insulin sensitivity, reducing the risk of *atherogenesis*, oxidative stress, and inflammatory states of the body[3]. The inflammatory process is part of the immune response (immune system) needed in certain conditions. The inflammatory mechanism helps eliminate damaged cells and accelerates healing[10].

Intermittent fasting reduces the body's inflammatory status by inhibiting the expression of proinflammatory cytokines.and chemokines, especially *interleukin-6 (IL-6)*[11]. *TNF-α* as a proinflammatory cytokines has a very important role in maintaining immunity from various external and internal factors conducing inflammation. Intermittent fasting affects the body's inflammatory status, which is characterized by inhibiting the expression of *cytokines* (especially IL-6), chemokines, and other inflammatory mediators that contribute to the pathogenesis of se*veral proinflammatory disorders such as atherosclerosis, insulin resistance, cardiovascular disease, cancer, polytrauma, and multiple organ dysfunction syndrome (MODS)[12–16].

IL-6 is a powerful pleiotropic cytokine regulating cell growth, differentiation and plays an important role in the immune response. *IL-6* is secreted by T cells, macrophages, osteoblasts, blood vessels, smooth muscle cells in tunica media. *IL-6* as a powerful proinflammatory cytokines.and immune-regulator plays an important role in leukocyte activation and migration, fever, acute phase response, cell proliferation, differentiation, and apoptosis[7].

According to the results of the above review, intermittent fasting does not only regulates the body's biochemical and physiological processes, but also elicits strong anti-inflammatory responses in both human and animal models. Intermittent fasting regulates the expression of proinflammatory cytokines, chemokines, and other proinflammatory mediators. However, further research is needed to clarify the molecular mechanism of the intermittent fasting protection signal function to consider this practice as a complementary therapeutic approach in the treatment of inflammatory disorders. This study aims to examine the effect of intermittent fasting on *IL-6* genes mRNA expression.

2. Method

This study is an experiment with a Pretest-Posttest with Control Group design to examine the effect of intermittent fasting on *IL-6 genes mRNA expression*. The subjects of this study were 10 mice divided into 2 groups: the intermittent fasting group and the control group. The research was carried out at the Laboratory of Molecular Biology and Immunology Department, Faculty of Medicine, Universitas Hasanuddin, Makassar, as a place for the maintenance and care of experimental animals as well as examining the *IL-6 genes mRNA expression*. The study was conducted for 30 days. Inclusion criteria: male mice strain BALB / c; albino mice; body weight of mice 25-35 grams; aged 6-9 weeks, healthy, characterized by active (nocturnal) movements, eyes clear, average rectal temperature of 37oC, and the fur is thick, smooth, shiny and clean. Exclusion criteria: the sick mice or showing signs of physical abnormalities. Research Instrument: Cages, food containers and standard D12102C feed ingredients, water bottles, wire netting for enclosure covers.

Before being treated, the mice were adapted first for 1 week so that the physical and psychological conditions of the mice were stable. Then mice were divided into two groups: Group I: Intermittent fasting mice 13 hours + standard diet as a treatment group, Group II: Mice with no intermittent fasting treatment + standard diet as controls. Mice in groups I and II, the blood samples were taken as much as 100 µl at pretest and posttest. The treatment is carried out for 30 days. Intermittent fasting for group I mice from 17:30 pm - 06.30 am (13 hours at night).

Measurement of IL-6 genes mRNA expression

Extract mRNA and measurement gene expression by quantitative real time PCR according to previous study.[17–19] Measurements were made at the Laboratory of Molecular Biology and Immunology Department, Faculty of Medicine, Universitas Hasanuddin, Makassar as a place to examine the expression of mRNA Genes IL-6 with Real Time PCR due to high sensitivity and specificity. The statistical test used was *Paired Sample T-Test* with the confidence degree of 95% and the value of a <0.05. All data were analyzed using Statistical Package for the Social Sciences (SPSS) Version 22.0 program (SPSS, Inc. Chicago).

3. Result

Table 1 shows that the intermittent fasting group gained weight evenly. Whereas in the control group there was 1 mice experienced weight loss of 1.1 g, from 31.5g to 30.4g. There was also one mice experienced a sharp increase in body weight of 6.9 g from 30.4 g to 37.3 g.

Table 1. Changes in Body Weight of Mice

No	Group	Body Weight		
		Pretest	**Posttest**	Δ
1	Control	32.9	33.8	0.9
2		31.5	30.4	-1.1
3		31.1	33.8	2.7
4		30.4	37.3	6.9
5		29.6	31.0	1.4
6		30.8	33.3	2.5
7		29.4	31.8	2.4
8	*Intermittent fasting*	29.0	34.6	5.6
9		28.1	30.5	2.4
10		29.7	34.1	4.4

Table 2. Effect of Intermittent Fasting on Decreased *IL-6* gene mRNA Expression in Mice

Group	Pretest	Posttest	ρ*	Δ	ρ*
	Mean + SD	**Mean + SD**			
Control	12.27 ± 0.81	12.29 ± 0.77	0.843	0.02 ± -0.05	0.003
Intervention	11.96 ± 0.59	8.12 ± 0.89	0.002	-3.84 ± 0.83	

IL-6 gene mRNA expression of the control group mice at an average pretest of 12.27 and at an average posttest of 12.29 with a mean difference of 0.81 at the pretest and 0.77 at the posttest, values ρ = 0.843 (ρ> 0, 05) showed that there was no difference in *IL-6* mRNA expression of mice in pretest and posttest. *IL-6* gene mRNA expression in mice in the intermittent fasting group was an average of 11.96 (pretest) and an average of 8.12 (posttest) with a mean difference of 0.59 (pretest) and 0.89 (posttest), the value of ρ= 0.002 (ρ <0.05) showed that there is a significant difference in the expression of IL-6 mRNA genes in mice (Table 2). The results of *Paired Sample T-Test* is ρ= 0.003 (ρ <0.05) at 95% confidence level indicate that there is a significant effect of intermittent fasting on the decline *in IL-6* gene mRNA expression in mice (Table 2).

4. Discussion

This study is an experimental study with a pretest-posttest with control group design to examine the effect of intermittent fasting on decreased *IL-6* mRNA genes expression. The subjects of this study were 10 mice as experimental animals which were divided into 2 groups: the treatment group and the control group. In the pretest, *IL-6* gene mRNA expression was examined in both groups of experimental animals. Within one month (30 days) then re-examination was carried out both

in the control group and in the intermittent fasting group. Examination of *IL-6* gene mRNA expression is measured by the Real Time PCR method due to high and specific sensitivity.

Samples were 10 male albino mice (mus musculus) which were divided into 5 individuals for the control group (not satisfied) and 5 individuals for the treatment group (satisfied). The mice is become experimental animal because it has calmer nature than the other one, especially when taking blood samples for examination using the Real Time PCR method. The whole sample is male mice with the reason that male mice do not undergo estrous cycles so that the sample becomes homogeneous, easily controlled and the results are expected to be more accurate. The sample used was 8-week-old whole mice for uniformity in both the control and treatment groups.

The average body weight of mice at pretest was 31.10 g for the control group and 29.40 g for the intermittent fasting group. The average body weight increased for both the control group and the intermittent fasting group at the posttest of 33.26 g (control group) and 32.86 g (intermittent fasting group). In the intermittent fasting group as a whole experienced weight gain evenly. Whereas in the control group there was 1 mouse experienced weight loss from 31.5 g to 30.4 g. There was also one mouse experienced a sharp increase in body weight from 30.4 g to 37.3 g.

The results of P*aired t-test* analysis showed that $\rho = 0.826$ ($\rho > 0.05$) means that there is no effect of intermittent fasting on the body weight of mice, this is indicated by the absence of significant differences between the body weight of mice in the control group and the intermittent fasting group. This means that in general weight changes that occur in intermittent fasting mice are not much different from changes in body weight in control mice. Intermittent fasting in this case becomes positive in order to balance and maintain a controlled weight gain. Based on Marice Sihombing (2010) normal body weight of mice at 12 weeks is in the average of 32.96 g, so that in general the body weight of mice in this study for both group were in the reference range, but specifically the intermittent fasting group is closer to the reference of 32.86 g (standard = 32.96 g).

Effect of intermittent fasting on decreased *IL-6* gene mRNA expression.

The mean value of IL-6 gene mRNA expression in intermittent fasting group subjects decreased from 11.96 in pretest to 8.12 at posttest. From these results it shows a decrease of 3.84 (19.12%). Strengthened from the test results, the value of $\rho = 0.002$ ($\rho < 0.05$) shows that there is a significant decrease in *IL-6* gene mRNA expression in subjects treated of intermittent fasting 13 hours per day for 30 days. Whereas the control group actually experienced an increase in IL-6 gene mRNA expression values of 0.02 (0.1%) from pretest 12.27 to 12.29 at posttest. This means that there is a 20-times decrease in *IL-6* gene mRNA expression in the intermittent fasting group compared to the control group.

Paired Sample T-Test results shows that $\rho = 0.003$ ($\rho < 0.05$) at the 95% confidence level means that there is a significant effect of intermittent fasting on the decrease in IL-6 gene mRNA expression in mice. From the foregoing shows that intermittent fasting can decrease the value of IL-6 gene mRNA expression significantly. This is consistent with the opinion of Arumugam et al. that intermittent fasting coordinatively increases levels of protective proteins and decreases proinflammatory cytokines. Production of IL-6, a cytokine that is involved in nerve degenerative processes is suppressed through intermittent fasting, thus protecting neurons against ischemic injury. In addition, intermittent fasting reduces oxidative stress and inflammation in different body tissues, including the brain. Low levels of reactive oxygen species (ROS) and Nuclear Factor kB (NF-kB) expression are inhibited, signaling a reduction in cytokine levels. NF-κB is the main proinflammatory signal pathway that regulates the expression of proinflammatory cytokines, such as IL-1β, IL-6, and TNF-α[20,21].

That is strengthened by the study results Akrami et al revealed that there were extraordinary differences between total cholesterol levels, FBS, triglycerides and LDL before and after intermittent fasting. The results showed that among the hematologic parameters, only platelet counts were very different before and after intermittent fasting.

The results also showed a decrease in proinflammatory cytokines CXC levels but also homoeostatic levels that did not change. Conclusions from the results of the study revealed that intermittent fasting is safe for normal healthy adults and is very beneficial in reducing cholesterol and triglycerides due to dyslipidemia. It is also possible to conclude that intermittent fasting is important in controlling inflammation through chemokines[11].

Intermittent fasting increases life expectancy. This is supported by research results that intermittent fasting effectively slows the aging process and protects the mice's heart against induced inflammation and fibrosis duration by inhibiting oxidative damage and activation of NF-kB[22]. Stroke in animals shows mortality due to focal ischemic stroke increases with age and decreases with intermittent fasting. Intermittent fasting has been shown to extend life expectancy and reduce inflammation and promote cancer in experimental animal models[2,23]. In this study, life expectancy increased by reducing inflammation by 20-times after intermittent fasting for 30 days.

Intermittent fasting has been shown to extend life expectancy and reduce inflammation and promote cancer in experimental animal models. Intermittent fasting can positively influence the inflammatory state characterized by decreased mRNA expression of the IL-6 gene as a significantly lower proinflammatory cytokines. Immune cells decline significantly but remain in the reference range. These results indicate that intermittent fasting reduces the body's inflammatory status by suppressing the expression of proinflammatory cytokines[20,24].

According to the results of the above review, intermittent fasting does not only regulates the body's biochemical and physiological processes, but also elicits strong anti-inflammatory responses in both human and animal models. Intermittent fasting has a significant anti-inflammatory effect and can be a complementary therapeutic approach in the treatment of inflammatory disorders. Decreased IL-6 gene mRNA expression (proinflammatory cytokines), chemokines, and other proinflammatory mediators by intermittent fasting is a sign that health is maintained[7]. From the results mentioned above, it can be concluded that intermittent fasting succeeded in reducing the rate of inflammation by 22 times after undergoing intermittent fasting for 30 days.

5. Conclusion

A decrease in IL-6 gene mRNA expression after intermittent fasting. The need for further research to determine the role of intermittent fasting in reducing the *IL-6* gene mRNA expression in humans.

Author's Contribution

All authors have contributed to the final manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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Ethics Approval

This study has obtained approval of research ethics eligibility from the Biomedical Research Ethics Commission in Experimental Animals, Faculty of Medicine, Universitas Hasanuddin, Makassar, with number of Recommendations for Ethical Approval Number 689/H4.8.4.5.31/PP36-KOMETIK/2018.

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