Original Article

The Effect of Moderate Intensity Exercise on the Secretion of IL-8 and TNF-α In Saliva as an Effort to Prevent Chronic Inflammation in Smokers

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ABSTRACT

Background

Cigarettes which contain dangerous substances that trigger chronic inflammation is one of highest problem in Indonesia. Long-term smoking can disrupt the immune system and increase the risk of non-communicable diseases for example cancer. Moderate-intensity exercise, like swimming, is believed to positively assist to reducing inflammation and enhancing quality of life. Further researches are necessary to understand the beneficial impact of exercise in preventing chronic inflammation in smokers. Early detection through analysis of IL-8 and TNF-α secretion in saliva is a potential focus in efforts to prevent chronic inflammatory conditions.

Objective

This research is intended to assess the impact of moderate-intensity exercise on the salivary secretion of IL-8 and TNF-α as a preventive measure against chronic inflammation in smokers.

Methods

Select research subjects who are healthy and given swimming training to measure maximum work capacity. Then the research subjects were divided into 4 groups, carried out a pretest before being given swimming treatment and a posttest after swimming by taking saliva samples using the gargling method for 2 minutes. Next, sample processing and measurement of IL-8 & TNF-α secretion. Then data analysis is carried out.

Results

There were significant differences between all group in IL-8 secretion (p=0.000 and p=0.005), also different in TNF-α secretion (p=0.160 and p=0.144). ***Conclusion:*** Moderate intensity exercise can decrease saliva secretion of IL-8 and TNF-α, so that can used to prevent chronic inflammation in smokers.

Keywords

moderate intensity of exercise; swimming; cigarettes; chronic inflammation; good health and well being

INTRODUCTION

Cigarettes are a controversial product that is widely circulated in society, but there are pros and cons to their use. Indonesia exhibits the highest incidence of smokers globally, ranking third after China and India, according to the Ministry of Health of the Republic of Indonesia (2018). According to the 2018 Riskesdas data[1], the prevalence of smokers in Indonesia is (33.8%), with a prevalence of men (62.9%) and women (4.8%). Based on surveys, Indonesian people already know the dangers contained in cigarettes but continue to smoke without caring about their health[2,3]. Both compounds in cigarettes and the smoke from it are almost toxic to the human body[4]. Cigarettes are one of the oxidative stress source[27].

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Studies on smoking indicate that smokers usually start their habit at a young age and find it difficult to stop[5]. Prolonged cigarette consumption can affect the immune-inflammatory system and result in various diseases related to chronic inflammation through the release of inflammatory cells into the bloodstream and the elevation of inflammatory markers like acute phase proteins and pro-inflammatory cytokines[6].

In the oral cavity, smoking may result in lesions or pathological conditions such as caries, oral cancer, precancerous lesions, and periodontal disease. Chronic inflammation can trigger an excessive reaction of Interleukin 8 (IL-8) and Tumor Necroting Factor α (TNF-α) which causes DNA damage, triggers cell death, and angiogenesis[8]. Angiogenesis refers to the biological mechanism via which fresh blood vessels are generated from currently present vessels. If this condition is not controlled properly, it can trigger chronic inflammation. The angiogenesis process is stimulated by various types of cells, including mast cells, fibroblasts, and macrophages. Macrophages can secrete angiogenic factors and cytokines, such as IL-8 and TNF-α, to initiate the process of angiogenesis. The increased amount of secretion of pro-inflammatory mediators around the tissue triggers activated macrophages to massively damage the tissue, which ultimately results in chronic inflammation[9].

Preventive measures need to be taken to prevent chronic inflammation, one of which is exercise. Several studies have proven the positive impact of exercise on the body's systems, including reducing inflammation, inhibiting the growth of cancer cells, increasing the working capacity of the heart and lungs, reducing blood glucose levels, reducing body fat levels, and increasing body immunity[10]. Based on intensity, exercise is divided into light, moderate, submaximal and maximal. Exercise can be divided depending on intensity, heart rate percentage, maximum work capacity and O_2 volume maximum[11]. Effective exercise can be started with moderate intensity and done regularly and the sport that can be done is swimming[10]. Swimming is an aerobic activity that functions to reduce chronic inflammation through anti-inflammatory effects and improving the immune system. Swimming can also increase insulin sensitivity, prevent obesity, relax the mind, and improve a person's quality of life[12, 26].

The objective of this study is to demonstrate the impact of moderate-intensity exercise on the release of IL-8 and TNF-α in saliva as an early detection measure and a preventive effort against chronic inflammatory conditions in smokers.

MATERIAL AND METHOD

This study utilizes a laboratory-based experimental design with a randomized pre-test and post-test control group approach, and has been granted approval by the Dental Research Ethics Committee of the Faculty of Dentistry, Airlangga University, Surabaya with number 0416/HRREC.FODM/IV/2024. The research was performed for approximately 4 – 5 months. Giving moderate intensity exercise (swimming) to the subjects was carried out at the Club Suryanaga Surabaya Swimming Pool, saliva analysis was performed at the Research Center of the Faculty of Dental Medicine, Airlangga University. The research subjects were students from the Faculty of Pedagogy and Psychology, Physical Education Study Program, PGRI Adibuana University, Surabaya. The number of subject for each group were 7, so the total research subjects used were 28 people.

The inclusion criteria for research subjects used were: 1) male gender, 2) aged 17-19 years, 3) healthy (no history of disease), smoking for at least 2 years and consuming 3 cigarettes per day.

Next, the research subjects were categorized into 4 distinct groups, specifically: 1) Negative control group (K1), a group of individuals who did not smoke and not treated with exercise; 2) Positive control group (K2) is a group of individuals who smoke, but are not treated with exercise; 3) Treatment group 1 (K3) is a group of individuals who do not smoke and are treated with exercise; 4) Treatment group 2 (K4) is a group of individuals who smoke and are treated with exercise.

Moderate intensity exercise is exercise with a maximum work capacity of 50-70%. In this study, swimming was chosen with a duration of 7 minutes each session, a frequency of 3 times per week, and a total duration of 12 weeks. IL-8 secretion was measured through salivary fluid, which was previously centrifuged and added with IL-8 antibodies. Measurements were then continued using the Human Interleukin IL-8 BT LAB ELISA Kit. TNF-α secretion can be measured through salivary fluid, which is previously centrifuged and the addition of TNF-α antibodies. Measurements were then continued using the Human Interleukin TNF-α BT LAB ELISA Kit.

Tools and materials

The tools utilized in this research compromise: stopwatch, sterile polypropylene tube, incubator 37°C±0.5°C' precision pipette and disposable pipette tip, Microplate reader (wavelength filter 450 ± 10nm), Centrifuge, ELISA Kit Human Interleukin IL-8 BT Lab, ELISA Kit Human Interleukin TNF-α BT Lab.

The research materials used were: saliva samples from each research subject, materials for examining IL-8 and TNF-α secretion in the BT Lab Human ELISA Kit, namely standard solution (8000pg/mL), pre-coated ELISA plate, Streptavidin- HRP, stop solution, standard diluent, wash buffer concentrate (25x), substrate solution A, substrate solution B, Biotinylated Human TNF-α antibody, Biotinylated Human IL-8 antibody.

Preparation of Subjects and Providing Treatment

An explanation regarding research implementation procedures is carried out before the research and if the subject agrees, then the subject is asked to fill out informed consent. Next, swimming training was given to research subjects by a professional swimming coach from the Suryanaga Swimming Club, Surabaya, for 3 days on Monday, Wednesday and Friday in one week. On the last day of training (day 3), maximum work capacity (MWC) was measured, namely the maximum time the research subjects swam until they felt tired. The research subjects were divided into 4 groups, then the treatment groups (K3 and K4) were given moderate intensity swimming, namely a duration of 70% of the MWC, to be precise 7 minutes. Swimming training is given 3 times a week, for 12 weeks at the Suryanaga Swimming Club, Surabaya.

Saliva Sampling

Saliva samples were collected using the gargling method, namely the subjects were asked to gargle vigorously for 2 minutes, then 2 cc of saliva was collected in a sterile tube and measurements were taken. Saliva samples were collected twice, namely pre-test (before being given exercise treatment) and post-test (24 hours after being given the last exercise treatment).

Saliva Sample Processing and Measurement IL-8 and TNF-α Level

The saliva samples were centrifuged at 2000-3000 revolutions per minute (RPM) for 20 minutes. The resulting liquid portion, the supernatant, was carefully collected without any leftover material. Samples, standard solutions, and reagents were prepared in accordance with the instructions. The reagent is brought to room temperature and tested. The quantity of indicator strips is chosen for testing purposes. Strips are placed into the frame in consideration of utilization, and any strips that have not been used should be stored at a temperature range of 2-8°C. A volume of 50 ul of the standard solution was poured into the standard well, noting that antibodies were intentionally excluded from the standard well due to the presence of biotinylated antibodies in the standard solution. First, add 40 ul of the sample solution to the sample well. Next, add 10 ul of IL-8 to the sample well. Then, add 50 ul of streptavidin-HRP to both the sample well and the standard well. Thoroughly combine the ingredients. A sealer was applied to the plate, which was then incubated at a temperature of 37°C for 60 minutes. The sealant was carefully extracted, and the plate was rinsed five times using a wash buffer. Each well was then immersed in 300 µl of wash buffer for a duration of 30 seconds to 1 minute per wash. To do automatic washing, remove liquid from each well by aspiration or decantation, and then rinse each well five times using a washing buffer. Wipe the plate using a paper towel or another material that can soak up liquid.

50 ul of substrate A solution was poured into each well, followed by the addition of 50 ul of substrate B solution to each well. Place the covered plate with a new sealer in a controlled environment at a temperature of 37°C for a duration of 10 minutes, ensuring no light exposure. Upon the addition of 50 ul of Stop Solution to each well, the blue color will promptly transition to a yellow hue. The optical density (OD) value of each well was measured within ten minutes following the application of the stop solution, using a microplate reader set to 450 nm. Measurement of IL-8 levels using Human Interleukin BT LAB ELISA Kit. The same procedure was carried out to measure TNF-α levels in saliva. Measurement of secretions TNF-α using Human TNF-α BT LAB ELISA Kit.

Data analysis

The data that was collected was further analyzed using the Shapiro-Wilk test to see if the research variables reflected a normal distribution. Homogeneity test to test whether the data between groups has a homogeneous covariance matrix or not using the Levene test. Parametric test if the data is normal and homogeneous using the Two-Way Anova Test, if there are significant

data differences, continue with the LSD difference test.

Ethical clearance

This study has been granted approval by the Dental Research Ethics Committee of the Faculty of Dentistry, Airlangga University, Surabaya with number 0416/HRREC.FODM/IV/2024.

RESULT

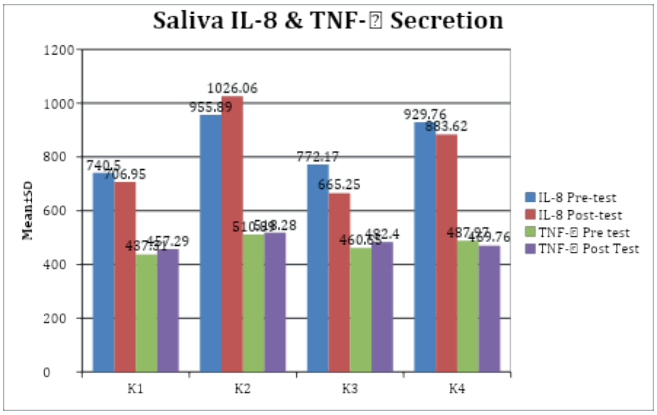

Figure 1. Pre-test and Post-test salivary IL-8 secretion in 4 groups.

Table 1. Results of pre-test and post-test differences in salivary IL-8 secretion using the One-Way Anova test

Group	n	Pre-test (significancy = p)	Post-test (significance = p)
K1	7	p = 0,000*	p = 0,005*
K2	7		
K3	7		
K4	7		

Note: * : there is a significant difference

Table 2. Test results differ between IL-8 secretion pre-test and post-test by LSD test

Group	K1	K2	K3	K4
K1		0,003* 0,000*	0,635 0,484	0,008* 0,006*
K2	0,003* 0,000*		0,010* 0,000*	0,695 0,023*
K3	0,635 0,484	0,010* 0,000*		0,025* 0,001*
K4	0,008* 0,006*	0,695 0,023*	0,025* 0,001*	

Note: * : there is a significant difference

Black color : pre-test

Red color : post-test

The research findings indicate an imbalance in the mean before and after the intervention in IL-8 secretion. The group that smoked and received moderate intensity exercise (swimming) experienced a reduction in IL-8 secretion. Meanwhile, the group that smoked and did not exercise experienced an increase in IL-8 secretion (figure 1).

The One-Way Anova difference test results presented in tables 1 indicate a statistically significant difference in IL-8 secretion before and after the intervention, with p-values of 0.005 and 0.000. Therefore, it was continued with the LSD test to determine the distinct groups. There are notable distinctions between groups K1 and K2, K1 and K4, K2 with K3, and K3 and K4, according to the LSD test findings in the pre-test group (table 2). Meanwhile, in the post-test group (table 2), significant differences occurred between groups K1 and K2, K1 and K4, K2 and K3, K2 and K4, and K3 and K4.

Table 3. Difference test results pre-test and post-test TNF-α secretion with One-Way Anova test

Group	n	Pre-test (significancy = p)	Post-test (significancy = p)
K1	7	p = 0,160	p = 0,144
K2	7		
K3	7		
K4	7		

Note: * : there is a significant difference

The research findings indicate an imbalance in the mean outcomes before and after the intervention in the secretion of TNF-α. The group that smoked and received moderate intensity exercise (swimming) experienced a decrease in TNF-α secretion. Meanwhile, the group that smoked and did not exercise experienced increased secretion of TNF-α (figure 1).

The One-Way Anova test findings, with p = 0.144 and p = 0.160, indicated no significant statistical differences before and after the intervention (table 3).

DISCUSSION

Smoking in Indonesia has become part of daily activities. Based on the Central Statistics Agency[13], smoking among teenagers or young adults in Indonesia

in 2022 is quite high with a higher percentage of men than women. Adolescence is a vulnerable age because of the high level of curiosity and search for identity as well as the tendency to be easily influenced by the surrounding environment. Several studies show that the majority of smoking habits start when they are teenagers, namely 15-19 years[14].

Cigarettes contain toxins which have an immune modulating effect and increase morbidity and mortality for the body due to the induction of cancer. Cigarette consumption causes chronic inflammation on mucosal surfaces, changes the host's response to exogenous antigens, and on immunity can be in the form of pro-inflammatory induction or suppressive effects as well as disrupting the body's innate reaction to pathogens, modulating the presentation of antigens, and inducing autoimmunity.

Inflammation is an vital component of the body's immune system reaction, and it can manifest as either acute or chronic. The inflammatory process begins when the body's immune system identifies and eliminates foreign and dangerous stimuli to begin the healing process. Chronic inflammation is inflammation that is slow and lasts for an extended duration, ranging from several months to even years. In most cases, the consequences of persistent inflammation differ based on the underlying source of the harm and the body's capacity to heal and overcome it. The primary cause of the majority of chronic diseases and a significant risk to an individual's well-being is the progressive development of chronic inflammation. Chronic inflammation can cause various diseases, like cardiovascular disease, chronic obstructive pulmonary disease (COPD), diabetes, asthma, chronic kidney disease, cancer, Alzheimer's disease, and inflammatory bowel disease[15,16].

Chronic inflammation can be caused by: (1) the immune system's inability to eliminate pathogenic organisms that induce acute inflammation, such as Mycobacterium Tuberculosis, Fungi, protozoa, and other infectious agents; (2) long-term exposure to certain irritants or foreign bodies; (3) autoimmune disorders that cause the immune system to recognize normal components of the body as foreign antigens, so that healthy tissue is damaged and causes certain diseases; (4) defects in cells that function in the inflammatory process cause inflammation to become persistent or recurrent; (5) the induction of biochemicals that lead to mitochondrial dysfunction and oxidative stress includes the elevated generation of free radical molecules, uric acid (urate) crystals, homocysteine, advanced glycation end products (AGEs), oxidized lipoproteins, and other substances[15].

Meanwhile, several factors can predispose to the occurrence of chronic inflammation, including: (1) age, which is positively linked with raised levels of various inflammatory molecules, can be caused by mitochondrial dysfunction or the accumulation of free radicals over a long period of time; (2) obesity, primarily caused by the production of adipokines and inflammatory mediators from fat tissue, which functions as an endocrine organ. Numerous studies have demonstrated a direct correlation between a person's body mass index and the quantity of pro-inflammatory cytokines released; (3) Diets high in saturated fats, trans fats, or refined sugars are associated with the production of pro-inflammatory molecules, especially in individuals with diabetes or those who are overweight; (4) smoking is associated with a decrease in the production of anti-inflammatory molecules and an increase in inflammation; (5) lower levels of sex hormones, such as testosterone and estrogen, can hinder the production and release of various pro-inflammatory cytokines; (6) stress and sleep disorders are connected, with stress being associated with the release of pro-inflammatory cytokines, while sleep disorders serve as an independent risk factor for chronic inflammation.

Smoking is the activity of smoking and inhaling smoke produced from burning tobacco packaged in cigarettes. Smoking is a habit that is detrimental to the body. According to the World Health Organization (WHO), several diseases are brought on by secondhand smoke from cigarettes in the environment, in active and passive smokers, both of which have very detrimental impacts on human health and the environment[17]. Smoking can cause pathological problems in the oral cavity in addition to its systemic consequences. Smoking can harm the oral cavity's soft tissues, including teeth. The damage caused, such as periodontal disease, dental decay, tooth loss, gingival recession, precancerous lesions, and cancer in the oral cavity.

The effect of smoking on inflammatory conditions can be observed, one of which is through the secretion of IL-8. IL-8 has an adaptive function in the acute inflammatory response by recruiting and activating monocytes and neutrophils at the site of inflammation. IL-8 is a chemokine from the CXC group which is actively

produced by monocytes, endothelium, epithelium and smooth muscle in the airways. Systemic production of IL-8 in humans who smoke can be induced by several factors, namely stress, environment, steroids, chemical, and inflammatory signals. The elevated IL-8 secretion observed in smokers is attributed to oxidative stress induced alongside pro-inflammatory conditions, leading to a higher IL-8 secretion rate in smokers compared to non-smokers. The pro-inflammatory nature of IL-8 inhibits the growth of normal cells, whereas in cancer cells, IL-8 promotes cell division, invasion, and disruption of tumor suppression through the Nf-κB pathway.

The results of research conducted by researchers showed that IL-8 secretion in individuals who smoked, namely in the K2 (1026.06 ± 53.996) and K4 (820.47 ± 98.130) groups was higher than in the non-smoking group, namely K1 (706. 95 ± 154,057) and K3 (665.25 ± 109,313). This shows that exposure to cigarettes without moderate intensity exercise intervention and moderate intensity exercise intervention without smoking can stimulate the release of different proinflammatory cytokines. The increase in IL-8 levels in smokers saliva in this research was also linear with the results of previous studies, which compared smokers and non-smokers. Research conducted by Sahibzada et al.[18], states that tobacco in any form can cause oxidative stress in cells which is characterized by an increase in Malondialdehyde (MDA) in the cell structure. This results in a pro-inflammatory cycle that raises cytokine levels, which are characterized by a correlation between MDA levels and daily cigarette consumption. Similar results were also found in research conducted by Gonzalo et al.[19], where after individuals did moderate and high intensity exercise, an elevation in IL-8 levels ranging from 1.37 to 2.77 times compared to before exercise was observed. An increase in IL-8 secretion can occur the day after exercise and then returns to its original level.

Cigarette smoke which is accepted by the body as a toxic substance can activate an inflammatory response. This response results in increased IL-8 secretion caused by activation of Nuclear Factor κappa B (NF-κB) via IκB Kinase (IKK). The release of IL-8 is a response to various stimuli, such as stress, the environment, steroids, and inflammatory signals. This stimulation activates the NF-κB pathway, which in turn activates IL-8 secretion. This demonstrates the involvement of NF-kB in the inflammatory response triggered by cigarette smoke, enhancing the synthesis and secretion of IL-8 as well as elevating the levels of other chemokines, such as monocyte chemotactic protein-1 (MCP-1)[16].

During exercise, IL-8 is locally synthesized in muscles, this can be seen after exercise eccentric through the component due to a pro-inflammatory response. IL-8 works on two receptors, namely CRCX-1 and CRCX-2 which have a similar structure but have different antigens[20]. IL-8 via the chemokine receptors CXCR-1 and CXCR-2 activates signal transducer and activator of transcription-3 (STAT3) and β-catenin, which causes cell proliferation and angiogenesis. Angiogenesis refers to the biological mechanism via which fresh blood vessels are generated from currently present vessels. The angiogenesis process is stimulated by various types of cells, namely mast cells, fibroblasts and macrophages. Macrophages can release cytokines and angiogenic factors that have a high potential to start angiogenesis. IL-8 plays a separate involvement in angiogenesis compared to its proinflammatory function. The expression of CXRC-2 by microvascular endothelial cells is liable for the process of angiogenesis caused by IL-8. Furthermore, exercise stimulates the expression of CXCR-2 mRNA and protein in muscle vascular endothelial cells. This indicates that IL-8 produced by muscle has a localized effect, particularly in promoting angiogenesis[21].

IL-8, which contains a powerful chemotactic and neutrophil activation protein called neutrophil activating peptide 1 (NAP-1), is released into the circulation after long-term and intense exercise. These findings indicate that not only the duration but also the intensity of exercise is also important for IL-8 secretion[22]. IL-8 also has direct and indirect effects on angiogenesis and affects endothelial cells. Homeostatic conditions in this process can possibly prevent chronic inflammation.

TNF-α is a pro-inflammatory cytokine formed by macrophages and innate immune cells. Most of them are Antigen Presenting Cells (APCs) which play a role in the chemotactic process of activating adaptive immunity. They work along with interleukins, such as IL-1α, IL-1β, IL-6, and IL-8, to integrate innate and adaptive immunity. TNF-α secretion is found in chronic and acute inflammatory conditions. Smoking can stimulate the secretion of TNF-α which causes chronic inflammation that triggers tissue damage. With this inflammation, APC will respond by moving towards

the damaged tissue and producing TNF-α. Therefore, TNF-α in subjects who smoke will be higher compared to those who do not smoke.

The study's findings indicated that the secretion of TNF-α in the smoker group without swimming intervention (K2) was higher than the non-smoker group without swimming intervention (K1) and the TNF-α secretion in the smoker group with swimming intervention (K4) was lower than the smoking group without swimming intervention (K3). The increase in TNF-α secretion in smoking individuals who did not exercise is correlates with the research results by Verma et al.[23], namely that there was an increase in TNF-α cytokines in smoking individuals who were not given exercise intervention, as well as an increase in TNF-α secretion. In the K3 is correlates with the study results by Zuo et al.[24] which exposed an increase in TNF-α even up to 2 weeks after exercise. Meanwhile, the decrease in TNF-α secretion in the K4 group most likely occurred due to exercise intervention, correlates with the research results by Andarianto et al.[25] where moderate intensity exercise was able to increase the release of pro-inflammatory and anti-inflammatory cytokines and produce catecholamines, corticotropin-releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and the secretion of cortisol.

An important function of TNF-α is to initiate immunological responses. TNF-α affects almost all cell types and is involved in blood clotting, metabolism, growth, differentiation, and cell death. The cytokine TNF-α is trimeric in nature and is present in two forms, namely trans membrane (mTNF) and soluble (sTNF). Two TNF receptors, namely TNF receptor 1 (TNFR-1) and 2 (TNFR-2) have different ligands and are activated by TNF isoforms in different ways. Nonetheless, their actions and paths may seem counter-intuitive. All cell types of TNFR-1 have a death domain with signal proteins that link it to cytotoxic pathways and activate MAP kinases and nuclear factor of kappa B (NFκB). By suppressing cytotoxic signals, activation of TNFR-1 can reduce cell survival. TNFR-2 can activate several kinases and NFkB signals, but does not have a death domain. Although it can be induced in non-immune cells such as fibroblasts, TNFR-2 is mostly found in immune cells where it functions to regulate inflammation and immune responses.

The conclusion of this article is moderate intensity exercise (swimming) can decrease saliva secretion of IL-8 and TNF-α, so that can used as a methods to prevent chronic inflammation in smokers.

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