



The Expression Change of Mmp-8 and Collagen Type-2 Intracell in Lung Tissue Due to Electronic Smoke Exposure

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Abstract

The number of electronic smokers has increased annually. Exposure to an electronic cigarette will increase free radicals in the body and result in oxidative stress causing lung tissue damage. The severity degree of lung tissue damage caused by electronic smoke exposure depends on the duration of electronic cigarette smoke exposure, and will affect Matrix Metalloproteinase-8 and collagen type-2 in the cells. The study aims to understand the change degree of Matrix Metalloproteinase-8 and collagen type-2 in lung tissue due to electronic cigarette smoke exposure. This study applied the experimental method with a post control group design. The male Wistar rats were used as the animal models in this research to assess cell damage through the Matrix Metalloproteinase-8 expression and collagen type-2 in the lung tissue using immunohistochemical staining. Exposure to electronic smoke cigarettes was given to each group of animal models with the difference in amount and time duration. The expression of Matrix Metalloproteinase-8 indicated a significant increase due to electronic smoke exposure (ANOVA, $p=0.000$). Meanwhile the expression of collagen type-2 showed a significant decrease because of electronic smoke exposure (ANOVA, $p=0.000$). Besides, MMP-8 and collagen type-2 manifested relationship existence and strong impact ($r=0.948$, $p=0.000$). The negative impact of exposure to electric cigarette smoke causes increased expression of Matrix Metalloproteinase-8 and decreased expression of type-2 collagen in lung tissue.

Introduction

In recent years, the number of electronic cigarette smokers has significantly increased. It is caused by a lack of knowledge about the long-term effects caused by the electronic cigarette as well as a limited amount of research data indicating the negative impacts of long-term use of electronic cigarettes. Moreover, the rule regulating electronic cigarette use has become a growing controversy. (Cherng, Tam, Christine, & Meza, 2016. Most people assume electronic cigarettes as one of the ways to reduce addiction to tobacco cigarettes, and the electronic cigarette is considered safer because it only contains nicotine (Vardavas, Filippidis, & Agaku, 2015). However, for most people, the electronic cigarette is assumed as a way to legalize smokers to smoke in public

and working places, and it can negatively affect teenagers smoking (Martínez-Sánchez et al., 2015). People's perception of electronic cigarette use also influences the increasing number of smokers. The electronic cigarette, considered safe by some people, can influence teenagers to start smoking the electronic cigarettes (Amrock, Lee, & Weitzman, 2016). It has become more popular in society through a massive marketing system via several media such as television, print publication, radio, and the internet. The promotion budget of electronic cigarettes in the United States in 2012 had increased almost double the same budget in 2011. In the second quarter of 2013, it increased more than eight times compared to the second quarter of 2012 (Xu, Guo, Liu, Liu, & Wang, 2016).

The increasing number of electronic

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cigarette smokers does not only happen in several developing countries but also in developed countries. In 2011, the prevalence of electronic cigarettes in young adults (18-28 years old) was the highest compared to the other age groups, reaching 4.9%-7%, with all age groups were 0.6% to 6.2%. The user prevalence of electronic cigarettes in the United States in the adult age group also increased 2 to 4 times higher in 2012 (Jaber et al., 2018). Meanwhile, among senior high school students, the use of electronic cigarettes was approximately 1.5% which later consistently increased in 2014 was 13.4%. New Zealand also experienced an increasing number of electronic cigarettes among teenagers (14-15 years old), almost three times higher than 7% in 2012 to 20% in 2014 (Thrasher et al., 2016). In recent years, Australia also demonstrated an increase in electronic cigarette smokers. The adult age group reached twice, from 4% in 2013 to 9% in 2016. The scores were obtained from two groups which were active smokers had an increase from 18% to 31%, while the non-smokers had an increase from 2% to 5%. However, this is inversely proportional to the use of cigarettes which decreases every year (Jongenelis, Kameron, Rudaizky, & Pettigrew, 2019). Policies on tobacco control such as an increase in cigarette taxes, smoke-free laws, limiting cigarette advertisements, and normalizing the behavior of smokers in active smokers are the most influential factors in reducing the number of smokers. But all these things have not been applied to users of e-cigarettes (Voigt, 2015).

The increase in the use of e-cigarettes does not only occur in high-income countries but also countries with medium and low incomes. In developing countries, electronic cigarette has been used, both individually and in pairs, along with tobacco cigarette (Palipudi et al., 2016). In Greece and Qatar, more than 60% of electronic cigarette smokers also use tobacco cigarettes concurrently, while the electronic cigarette smokers originating from non-smokers have reached 35.6% in Greece and 15% in Qatar (Palipudi et al., 2016).

Electronic cigarettes are battery-powered cigarettes operating through the heating process of an element (metal coil) by evaporating propylene glycol solvent, vegetable

glycerin, and flavoring, which sometimes contain nicotine (Grana, Benowitz, & Glantz, 2014). Electronic cigarettes are considered to have fewer side effects on health than tobacco cigarettes. Besides, more modern packaging and better marketing strategies have made electronic cigarettes a lifestyle choice for smokers and teenagers (Canistro et al., 2017). The electronic cigarette is always claimed as one of the effective ways to stop smoking or as a substitute for cigarette because it does not contain tar, carbon monoxide, and other chemical compounds. This has led to an annual increasing rate of electronic cigarette smokers (Polosa et al., 2011).

Cigarette smoke contains several types of a high number of free radicals, estimated to reach more than 1016 for every inhalation, including Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) (Dellinger, Khachatryan, Masko, & Lomnicki, 2011). Reactive Oxygen Species (ROS) consist of superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\bullet}) as regular products of oxygen molecule reduction. Radical oxygen is not only produced by mitochondria but neutrophils and macrophages can also produce ROS through the plasma membrane (Reuter, Gupta, Chaturvedi, & Aggarwal, 2010), (Herlina, Riyanto, Martono, & Rohman, 2018). In Hypoxic conditions, mitochondria produce Nitric Oxide (NO), producing other Reactive Nitrogen Species (RNS), for example, aldehydes-malondialdehyde and 4-hydroxynonenal (Arulselvan et al., 2016). In a normal condition and with a balance between free radicals and antioxidants, the free radicals serve as the body's defense mechanism (Ravipati et al., 2012). A significant increase in the number of free radicals due to electronic cigarette smoke exposure can cause the occurrence of oxidative stress in lung tissue (Zhang et al., 2018). Oxidative stress is triggered by an imbalance between the number of free radicals entering the lung tissue and antioxidants in the body resulting in injury in all cellular components such as lipid, protein, and DNA causing cells' death (R. V. Suryadinata, Wirjatmadi, & Adriani, 2017), (Sagor, Reza, Tabassum, Rahman, & Alam, 2017). Some diseases can also be caused by cigarettes, such

as cancer, cardiovascular diseases, and Chronic Obstructive Pulmonary Disease (COPD) (Goel et al., 2015).

The number and size of particles generated from electronic cigarettes are the same as the ones produced by tobacco cigarettes. Even some electronic cigarettes can produce more particles compared to tobacco cigarettes (Grana et al., 2014). Particles produced from e-cigarettes irritate of the airways, so mucous hypersecretion occurs in the bronchi (R. V. Suryadinata, Wirjatmadi, & Adriani, 2016). The number increase of free radical particles can trigger inflammation reaction in the lung tissue (Pratiwi, Lorensia, & Suryadinata, 2018). Inflammation reaction is a lung defense mechanism against dangerous stimuli such as pathogens, cell damage, and harmful chemical compounds. Moreover, acute inflammation response can minimize injury or infection caused in the lung tissue. The inflammatory process changes blood vessel permeability, leukocyte movement, and the release of inflammatory mediators (Chen et al., 2018).

However, a prolonging inflammation process in airways can result in lung cell damage. It can cause cell lysis occurrence impacting deteriorating lung cell function (Levy & Serhan, 2014). In a pathological condition or cell damage, there is an increase in productivity and activity of Matrix Metalloproteinase-8, while collagen type-2 will experience an intracellular decrease (Asano et al., 2010). The change of Matrix Metalloproteinase-8 and collagen type-2 can trigger fibrosis formation in the lung. (McKleroy, Lee, & Atabai, 2013)

An electronic cigarette is always considered to contain fewer chemical compounds than tobacco cigarettes. The fact is that electronic cigarettes safety has not yet been proven, and the side effects of their long-term use on the lung tissue have not yet been known (Jensen et al., 2015; Suryadinata & Wirjatmadi, 2020). Thus verification of histology aspects of the levels of Matriks Metalloprotein-8 and collagen type 2 as a parameter of lung tissue damage due to the use of electronic cigarettes

in male Wistar rat models is required. These parameters can provide a direct picture of lung tissue damage compared to the use of malondialdehyde levels (Wirjatmadi & Suryadinata, 2020).

Methods

This study is an experimental research using a post-test control group design. The sample used is male Wistar rats (*Rattus novergicus*). This research was divided into six groups with different time duration of administration treatment of electronic cigarette smoke exposure for each. The smoke exposure was done for 5 minutes during each intervention administration. The differing aspect of each group was the total amount of administration per day and the time duration per week. The first group was the negative control group which was not exposed to electronic cigarettes and compared to treatment groups. While the rest, in each group, there was the exposure to electronic cigarette smoke for several times duration and an observation of lung tissue using immunohistochemical staining (HIS) was conducted to see the tissue damage.

Samples of experimental animals Wistar rats (*Rattus novergicus*) aged 2-3 months with a weight of 200-250 grams, move actively, macroscopically found no abnormalities and have never been the object of research. Before the treatment is carried out, all animals try to do the adaptation process first for 5-7 days. The study was conducted at the Laboratory of the Faculty of Medicine, Airlangga University, based on the 3R principle (Replacement, Reduce, and Refinement). Experimental animals were placed in cages measuring 800 cm² per 5 animals with ventilation and room temperature around 25oC. Cleaning the cage and providing drinking water are done periodically, and food is about 20-30 grams/day. Each group will be given exposure to electric cigarette smoke that is different in time, amount, and duration of administration following research procedures.

Sample replication using is used to compare between treatment groups

$$n = \left\{ \frac{(Z_{1-\alpha} + Z_{1-\beta}) \cdot \sigma}{\mu_1 - \mu_0} \right\}^2$$

Based on the calculation, the minimum sample in this study is 5 male Wistar rats in each group. The solution of the electric cigarette used in this study contained 6 mg of nicotine. The room size where the exposure to electric cigarette smoke measured is 50 cm x 40 cm x 20 cm. The room is passed through by a pipe, flowing e-cigarette smoke. Provision of exposure to cigarette smoke is adjusted to the length of administration planned in the study.

Samples were assessed semiquantitatively according to the modified Remmele method, where the Remmele scale index (Immuno Reactive Score / IRS) is the result of multiplying the percentage score of immunoreactive cells with the color intensity score on immunoreactive cells. The data for each sample is the average value of the IRS observed in 5 (five) Field View (LP) different at 1000x magnification. All of these examinations use a light microscope.

The male Wistar rats were divided into six groups, including negative control and treatment groups. The first as the negative control group was a group given no intervention for four weeks. In the second or treatment group I got the e-cigarette smoke intervention once every five minutes per day for a week. The third or the treatment group II got e-cigarette smoke exposure intervention twice every five minutes per day for a week. Treatment group III got the intervention of e-cigarette smoke exposure once every five minutes per day for two weeks. The treatment group IV got e-cigarette smoke

exposure twice every five minutes per day for two weeks. The last control group was given the intervention of e-cigarette smoke exposure once every five minutes per day for three weeks.

The data collected performed statistical tests using ANOVA test analysis with SPSS version 20 to see the difference between Metalloprotein 8 matrix and collagen type 2 in lung tissue in all groups. Then the Least Significant Differences (LSD) test is performed to compare between groups. In addition, a trial was conducted to see the existence of a relationship between the two groups. Data will be presented in average numbers from the Immuno Reactive Score (IRS)

Results And Discussion

The study were carried out by comparing the Metalloprotein 8 (MMP-8) matrix and the collagen type 2 average in each group per 5 visual fields. Based on Figure 1, the average value and Standard Deviation of the Metalloprotein 8 (MMP-8) matrix can be seen in each group. These results show the increasing Metalloprotein 8 (MMP-8) matrix in each group directly proportional to the length of exposure time to cigarette smoke. In group I, the mean value of the Metalloprotein 8 (MMP-8) matrix reached 2.00 ± 0.17 , which is the lowest mean value in all groups. While the highest mean value was obtained in group VI, reaching 7.48 ± 0.34 .

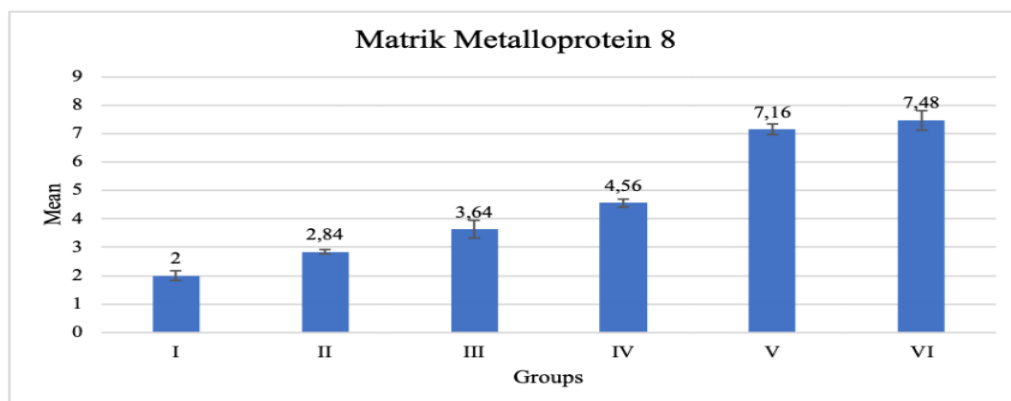


Figure 1. The Mean Value of Matrik Metalloprotein 8 (MMP-8) in Each Group

ANOVA analysis of the Metalloprotein 8 (MMP-8) matrix shows the difference in the Metalloprotein 8 (MMP-8) matrix in various groups ($p = 0,000$), then analyzed using Least Significance Different (LSD) to see the difference in Metalloprotein 8 (MMP-8) -8)

between groups (Table 1). Based on Table 1, there was a significant difference ($p < 0,05$) in the average Metalloprotein 8 (MMP-8) matrix between all groups, except group 5 and group 6 which showed no difference ($p > 0,05$).

Table 1. Least Significant Difference (LSD) test on Matrik Metalloprotein 8 (MMP-8) in Each Group

Groups	I	II	III	IV	V	VI
I	-	-	-	-	-	-
II	0,014	-	-	-	-	-
III	0,000	0,019	-	-	-	-
IV	0,000	0,000	0,008	-	-	-
V	0,000	0,000	0,000	0,000	-	-
VI	0,000	0,000	0,000	0,000	0,322	-

Based on picture 2, Shows the mean value of collagen type 2 is inversely proportional to the duration of exposure to cigarette smoke. In

group VI, type 2 collagen reached the lowest value of 2.84 ± 0.15 . The highest value was in the group I, namely 10.04 ± 0.75 .

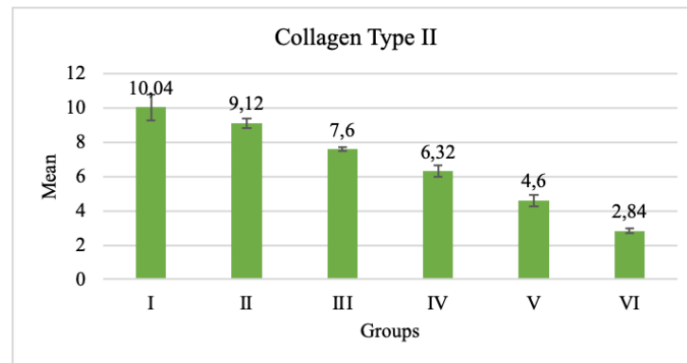


Figure 2. The Mean Value of Collagen Type 2 in Each Group

ANOVA analysis results on collagen type 2 showed differences in average collagen type 2 in various groups ($p = 0,000$), then analyzed using the least significance difference (LSD) to see differences in collagen type 2 between

groups (Table 4). Based on Table 2, there was a significant difference ($p < 0,005$) in collagen type 2 between the negative control group, and all treatment groups

Table 2. Least Significant Difference (LSD) Test Results on Collagen Type 2 in Each Group

Groups	I	II	III	IV	V	VI
I	-	-	-	-	-	-
II	0,012	-	-	-	-	-
III	0,000	0,003	-	-	-	-
IV	0,000	0,000	0,001	-	-	-
V	0,000	0,000	0,000	0,000	-	-
VI	0,000	0,000	0,000	0,000	0,000	-

The results of the correlation test analysis showed a strong relationship between Metalloprotein 8 matrix and collagen type 2

($r = 0.948$). In addition, the two groups had a significant relationship ($p < 0,05$) (Table 3).

This research indicates that the administration of electronic cigarette exposure will result in lung tissue damage. The group without administration of electronic cigarette smoke exposure does not show any lung tissue damage marked by the expression of a low level of Matrix Metalloproteinase-8 and a high level of collagen type-2. Meanwhile, the most severe lung tissue damage is exhibited in the group receiving 3-week exposure to cigarette smoke, where there is an increase of Matrix Metalloproteinase-8 and a decrease of collagen type-2.

Lung inflammation occurs because electronic cigarette exposure contains several harmful compounds entering the airways and can result in a free radicals increase in the body (R. V. Suryadinata, Lorensia, & Sari, 2017). The number increase of free radicals entering the body can cause antioxidant imbalance problems in the body. It can trigger the occurrence of lipid peroxidation, causing cells to undergo oxidative stress. The content of cigarette smoke can be divided into free radicals and non-radical oxidants. The free radical type that plays the most role in cigarette smoke is the superoxide anion ($O_2^{\bullet-}$). These free radicals can be directly neutralized by enzymatic antioxidants, the Superoxide Dismutase. The result is hydrogen peroxide (H_2O_2) which is a non-radical oxidant. Furthermore, the radical Hydrogen peroxide (H_2O_2) will be neutralized by enzymatic antioxidants Gluthation peroxidase (GSH-Px) and catalase to be converted into water (H_2O) and oxygen (O_2) (Karmaker, Lira, Das, Kumar, & Rouf, 2017). Radicals Hydrogen peroxide (H_2O_2) can also react again with superoxide anion ($O_2^{\bullet-}$) to hydroxyl radicals (OH^{\bullet}) called the Haber-Weiss reaction. In addition, if the Hydrogen Peroxide Radical (H_2O_2) reacts with pheton (Fe^{2+}) or known as the pheton reaction, it can also produce hydroxyl radicals (OH^{\bullet}). Increased hydroxyl radicals (OH^{\bullet}) in the body will aggravate the occurrence of lipid peroxidation. Malondialdehyde (MDA) is the final result most widely used to measure the increase in free radicals in the body (Marrocco, Altieri, & Peluso, 2017).

Lipid peroxidase undergone by the cells will cause cell rupture or necrosis, often called Damage-associated molecular patterns

(DAMPs) or more commonly known as cell debris (Virlando Suryadinata, 2018). Cell debris exiting the cell can disrupt the microenvironment and is regarded as a foreign object by the body. The reaction will improve macrophage activities to reach the cell debris and do the phagocytosis process, becomes one of the macrophage's roles as a non-specific immune system.

The phagocytosis process is carried out by macrophages with the help of lysosomes or better known as phagolysosomes. In the body, macrophages do not only act as a non-specific immune system. In addition, macrophages like Antigen Presenting Cells (APC) which can present a major histocompatibility complex (MHC) class I or class II and play a vital role in the adaptive immune system. The class I major histocompatibility complex (MHC) will be identified by cytotoxic CD 8+ T cells. The class II major histocompatibility complex (MHC) will be recognized by CD 4+ T cells (Wieczorek et al., 2017).

The phagocytosis of cell debris conducted by macrophages will trigger several types of inflammation mediators such as Interleukin 1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and Tumor Necrosis Factor- α (TNF- α) (Wojdasiewicz, Poniatowski, & Szukiewicz, 2014). Interleukin-8 has the role of stimulating neutrophil movement or more commonly known as Neutrophil Chemotactic Factor (NFC) to fight against a pathogen or foreign objects through recognition of several receptors (de Oliveira et al., 2013).

The Neutrophil increase will damage Matrix Metalloproteinase (MMPs). It is because Matrix Metalloproteinase (MMPs) is responsible for the maintenance of extracellular matrix (ECM) protein surrounding endothelial in the whole body. Besides, Matrix Metalloproteinase also has a role in the inflammation process, which will increase more inflammation process in the tissue. Moreover, matrix metalloproteinase (MMPs) serves to balance the homeostasis of several collagen types. One type of matrix metalloproteinase which is in the airways and has a role during inflammation in the lung tissue is matrix metalloproteinase-8 (MMP-8) (Basu, Donaworth, Siroky, Devarajan, & Wong, 2015).

Matrix Metalloproteinase-8 is initially called Neutrophil Collagenase because there were specific grains obtained in the neutrophil and is also expressed in epithelial cells, fibroblast, macrophage, and endothelial. Several studies also show the existence of MMP-8 activities in tumors and metastasis (Thirkettle et al., 2013). But later on, MMP-8 is linked with the inflammation process and fibrosis in the lung. It is because Matrix Metalloproteinase-8 directly impacts on collagen type-2 existing in the lung tissue. The expression of MMP-8 occurring due to inflammation reaction is influenced by the secretion of Interleukin 6 and Interleukin 8 as proinflammation cytokines (Rathnayake, Gieselmann, Heikkinen, Tervahartiala, & Sorsa, 2017).

Collage is the primary part of the extracellular matrix and contains a high protein level. Collagen in tissues also serves as a mechanical defense and organism. Besides, collagen can also serve as a signaling molecule for cellular shape and behavior. The body has 16 types of collagen. But the most prominent are collagen types I, II, and III. Collagen is produced by cells according to their morphology, distribution, function, and pathogenesis (Deshmukh, Dive, Moharil, & Munde, 2016). The type of collagen existing in the lung tissue is collagen type-2 and plays a role in fibrosis formation in the lung tissue. Damage to collagen type-2 in the lung tissue will result in cell damage as well as cell death. The content of collagen type-2 in the lung tissue influenced by MMP-8 will show a relationship exists that an increase of MMP-8 will cause a decrease of collagen type-2, hence causing lung tissue damage, further triggering fibrosis tissue formation in the lung (Pardo, Cabrera, Maldonado, & Selman, 2016).

Lung fibrosis is a pathological syndrome as a result of lung injury. The pathobiological mechanism of pulmonary fibrosis produces a different remodeling response in the lungs. Lung fibrosis is a chronic, progressive, and severe lung disease. It is because disruption of the Extracellular Matrix (ECM), which is irregular. The smoke of electronic cigarettes is one of the main factors of lung fibrosis occurrence. Besides, the reason of lung fibrosis is caused by job exposure, dust, and smoke in

motor vehicles (Awadalla, Hegazy, Elmetwally, & Wahby, 2012). Lung fibrosis disease is often associated with environmental disturbance (Chilosi, Poletti, & Rossi, 2012).

Conclusion

According to conducted research, it can directly provide some information related to the negative impacts caused by electronic cigarettes. Some misconceptions popular among people related to the safety of electronic cigarettes must be addressed properly as soon as possible. Perceptions viewing electronic cigarettes are safer than the tobacco cigarettes in reducing addiction to tobacco cigarette of active smokers must be reconsidered. This study has shown that the negative impacts of free radicals caused by electronic cigarette smoke exposure have directly influenced the intracellular lung tissue. The inflammation process contributes to lung tissue through some inflammation mediators. It will result in an intracell increase of Matrix Metalloproteinase-8, which later will reduce the collagen type-2 in the lung tissue.

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References

- Amrock, S. M., Lee, L., & Weitzman, M., 2016. Perceptions of e-Cigarettes and Noncigarette Tobacco Products Among US Youth. *Pediatrics*, 138(5), e20154306.
- Arulsevan, P., Fard, M.T., Tan, W.S., Gothai, S., Fakurazi, S., Norhaizan, M. E., & Kumar, S.S., 2016. Role of Antioxidants and Natural Products in Inflammation. *Oxidative Medicine and Cellular Longevity*, 2016, pp.1–15.
- Asano, K., Shikama, Y., Shoji, N., Hirano, K., Suzaki, H., & Nakajima, H., 2010. Tiotropium bromide inhibits TGF- β -induced MMP production from lung fibroblasts by interfering with Smad and MAPK pathways in vitro. *International Journal of Chronic Obstructive Pulmonary Disease*, 5, pp.277–286.
- Awadalla, N.J., Hegazy, A., Elmetwally, R.A., & Wahby, I., 2012. Occupational

- and Environmental Risk Factors for Idiopathic Pulmonary Fibrosis in Egypt: A Multicenter Case-control Study. *The International Journal of Occupational and Environmental Medicine*, 3(3), pp.107–116.
- Basu, R.K., Donaworth, E., Siroky, B., Devarajan, P., & Wong, H.R., 2015. Loss of Matrix Metalloproteinase-8 is Associated with Worsened Recovery After Ischemic Kidney Injury. *Renal Failure*, 37(3), pp.469–475.
- Canistro, D., Vivarelli, F., Cirillo, S., Marquillas, C.B., Lazzaretti, M., Laura, M., Paolini, M., 2017. *E-cigarettes Induce Toxicological Effects that Can Raise the Cancer Risk*, *National Center for Biotechnology Information*, 17(1), pp.1–9.
- Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., & Zhao, L., 2018. Inflammatory Responses and Inflammation-Associated Diseases in Organs. *Oncotarget*, 9(6), pp.7204–7218.
- Cherng, S.T., Tam, J., Christine, P.J., & Meza, R., 2016. Modeling the Effects of E-cigarettes on Smoking Behavior. *Epidemiology*, 27(6), pp.819–826.
- Chilosi, M., Poletti, V., & Rossi, A., 2012. *The Pathogenesis of COPD and IPF: Distinct Horns of the Same Devil?* *Respiratory Research*, 13(1), pp.3.
- de-Oliveira, S., Reyes-Aldasoro, C.C., Candel, S., Renshaw, S.A., Mulero, V., & Calado, Â., 2013. Cxcl8 (IL-8) Mediates Neutrophil Recruitment and Behavior in the Zebrafish Inflammatory Response. *The Journal of Immunology*, 190(8), pp.4349–4359.
- Dellinger, B., Khachatryan, L., Masko, S., & Lomnicki, S., 2011. Free Radicals in Tobacco Smoke. *Mini-Reviews in Organic Chemistry*, 8(4), pp.427–433.
- Deshmukh, S., Dive, A., Moharil, R., & Munde, P., 2016. Enigmatic Insight Into Collagen. *Journal of Oral and Maxillofacial Pathology*, 20(2), pp.276.
- Goel, R., Durand, E., Trushin, N., Prokopczyk, B., Foulds, J., Elias, R.J., & Richie, J.P., 2015. Highly Reactive Free Radicals in Electronic Cigarette Aerosols. *Chemical Research in Toxicology*, 28(9), pp.1675–1677.
- Grana, R., Benowitz, N., & Glantz, S.A., 2014. E-Cigarettes. *Circulation*, 129(19), pp.1972–1986.
- Herlina, N., Riyanto, S., Martono, S., & Rohman, A., 2018. Antioxidant Activities, Phenolic and Flavonoid Contents of Methanolic Extract of *Stelechocarpus burahol* Fruit and its Fractions. *Dhaka University Journal of Pharmaceutical Sciences*, 17(2), pp.153–159.
- Jaber, R.M., Mirbolouk, M., DeFilippis, A.P., Maziak, W., Keith, R., Payne, T., Stokes, A., Benjamin, E., Bhatnagar, A., Blankstein, A., Saxena, A., Blaha, M.J., & Nasir, K., 2018. Electronic Cigarette Use Prevalence, Associated Factors, and Pattern by Cigarette Smoking Status in the United States From NHANES (National Health and Nutrition Examination Survey) 2013–2014. *Journal of the American Heart Association*, 7(14).
- Jensen, R.P., Luo, W., Pankow, J.F., Strongin, R.M., & Peyton, D.H., 2015. Hidden Formaldehyde in E-Cigarette Aerosols. *New England Journal of Medicine*, 372(4), pp.392–394.
- Jongenelis, M.I., Kameron, C., Rudaizky, D., & Pettigrew, S., 2019. Support for E-cigarette Regulations Among Australian Young Adults. *BMC Public Health*, 19(1), pp.67.
- Karmaker, N., Lira, D.N., Das, B.K., Kumar, U., & Rouf, A.S.S., 2017. Synthesis and Antioxidant Activity of Some Novel Benzimidazole Derivatives. *Dhaka University Journal of Pharmaceutical Sciences*, 16(2), pp.245–249.
- Levy, B.D., & Serhan, C.N., 2014. Resolution of Acute Inflammation in the Lung. *Annual Review of Physiology*, 76(1), pp.467–492.
- Marrocco, I., Altieri, F., & Peluso, I., 2017. Measurement and Clinical Significance of Biomarkers of Oxidative Stress in Humans. *Oxidative Medicine and Cellular Longevity*, 2017, pp.1–32.
- Martínez-Sánchez, J.M., Fu, M., Martín-Sánchez, J.C., Ballbè, M., Saltó, E., & Fernández, E., 2015. *Perception of Electronic Cigarettes in the General Population: Does Their Usefulness Outweigh Their Risks?* *BMJ Open*, 5(11),

- e009218.
- McKleroy, W., Lee, T.-H., & Atabai, K., 2013. Always Cleave Up Your Mess: Targeting Collagen Degradation to Treat Tissue Fibrosis. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 304(11), pp.L709–L721.
- Palipudi, K.M., Mbulo, L., Morton, J., Mbulo, L., Bunnell, R., Blutcher-Nelson, G., Asma, S., 2016. Awareness and Current Use of Electronic Cigarettes in Indonesia, Malaysia, Qatar, and Greece: Findings From 2011–2013 Global Adult Tobacco Surveys. *Nicotine & Tobacco Research*, 18(4), pp.501–507.
- Pardo, A., Cabrera, S., Maldonado, M., & Selman, M., 2016. Role of Matrix Metalloproteinases in the Pathogenesis of Idiopathic Pulmonary Fibrosis. *Respiratory Research*, 17(1), pp.23.
- Polosa, R., Caponnetto, P., Morjaria, J.B., Papale, G., Campagna, D., & Russo, C., 2011. Effect of an Electronic Nicotine Delivery Device (e-Cigarette) on Smoking Reduction and Cessation : A Prospective 6-Month Pilot Study. *BMC Public Health*, 11.
- Pratiwi, S.R., Lorensia, A., & Suryadinata, R.V., 2018. Vitamin C and E Intake with SQ-FFQ towards Smokers' and Non-Smokers' Lung Function. *Media Kesehatan Masyarakat Indonesia*, 14(2), pp.101.
- Rathnayake, N., Gieselmann, D.-R., Heikkinen, A., Tervahartiala, T., & Sorsa, T., 2017. Salivary Diagnostics—Point-of-Care Diagnostics of MMP-8 in Dentistry and Medicine. *Diagnostics*, 7(1), pp.7.
- Ravipati, A.S., Zhang, L., Koyyalamudi, S.R., Jeong, S.C., Reddy, N., Bartlett, J., Smith, P.T., Shanmugam, K., Münch, G., Wu, M.J., Satyanarayanan, M., & Vysetti, B., 2012. Antioxidant and Anti-inflammatory Activities of Selected Chinese Medicinal Plants and their Relation with Antioxidant Content. *BMC Complementary and Alternative Medicine*, 12(1), pp.1192.
- Reuter, S., Gupta, S.C., Chaturvedi, M.M., & Aggarwal, B.B., 2010. *Oxidative Stress, Inflammation, and Cancer: How are They Linked? Free Radical Biology and Medicine*, 49(11), pp.1603–1616.
- Sagor, M.A.T., Reza, H.M., Tabassum, N., Rahman, M.M., & Alam, M.A., 2017. Fresh Bitter Melon Fruit (*Momordica charantia*) Attenuated Oxidative Stress, Fibrosis and Renal Injury in Carbon Tetrachloride Treated Rats. *Dhaka University Journal of Pharmaceutical Sciences*, 16(2), pp.205–214.
- Suryadinata, R.V., Wirjatmadi, B., & Adriani, M., 2016. Pengaruh Perubahan Hiperplasia Sel Goblet Selama 28 Hari Paparan Asap Rokok Dengan Pemberian Antioksidan Superoxide Dismutase. *The Indonesian Journal of Public Health*, 11(1), pp.60.
- Suryadinata, R.V., Wirjatmadi, B., & Adriani, M., 2017. Effectiveness Decrease Combined with Supplements Malondialdehyde Antioxidant Superoxide Dismutase Gliadin Melon with Due to Exposure to Cigarette. *Global Medical And Health Communication*, 5(2), pp.79–83.
- Suryadinata, R.V., Lorensia, A., & Sari, R.K., 2017. Differences in Nutrition Food Intake and Body Mass Index between Smoker and Non-smoker in Adult. *Indonesian Journal of Clinical Pharmacy*, 6(3), pp.171–180.
- Suryadinata, R.V. & Wirjatmadi, B., 2020. Selenium Linked to Increased Antioxidant Levels and Decreased Free Radicals in Lung Tissue of Wistar Rats Exposed to E-Cigarette Smoke. *Journal of Global Pharma Technology*, 12(9), pp.32–39
- Thirkettle, S., Decock, J., Arnold, H., Pennington, C.J., Jaworski, D.M., & Edwards, D.R., 2013. Matrix Metalloproteinase 8 (Collagenase 2) Induces the Expression of Interleukins 6 and 8 in Breast Cancer Cells. *Journal of Biological Chemistry*, 288(23), pp.16282–16294.
- Thrasher, J.F., Abad-Vivero, E.N., Barrientos-Gutierrez, I., Pérez-Hernández, R., Reynales-Shigematsu, L.M., Mejía, R., & Sargent, J.D., 2016. Prevalence and Correlates of E-Cigarette Perceptions and Trial Among Early Adolescents in Mexico. *Journal of Adolescent Health*,

- 58(3), pp.358–365.
- Vardavas, C.I., Filippidis, F.T., & Agaku, I.T., 2015. Determinants and Prevalence of E-cigarette Use Throughout the European Union: A Secondary Analysis of 26 566 Youth and Adults from 27 Countries. *Tobacco Control*, 24(5), pp.442–448.
- Virlando, S.R., 2018. Effect of Free Radicals on Inflammatory Process in Chronic Obstructive Pulmonary Disease (COPD). *Amerta Nutrition*, 2(4), pp.317–324.
- Voigt, K., 2015. Smoking Norms and the Regulation of E-Cigarettes. *American Journal of Public Health*, 105(10), pp.1967–1972.
- Wieczorek, M., Abualrous, E.T., Sticht, J., Álvaro-Benito, M., Stolzenberg, S., Noé, F., & Freund, C., 2017. Major Histocompatibility Complex (MHC) Class I and MHC Class II Proteins: Conformational Plasticity in Antigen Presentation. *Frontiers in Immunology*, 8.
- Wirjatmadi, B., & Suryadinata, R.V., 2020. The Alteration on Malondialdehyde Content on Wistar Rats' Blood and Lungs Tissue to Ward the Exposure of Electric Cigarette Smoke. *Indian Journal of Public Health Research & Development*, 11(3), pp.1881-1887.
- Wojdasiewicz, P., Poniowski, Ł.A., & Szukiewicz, D., 2014. The Role of Inflammatory and Anti-Inflammatory Cytokines in the Pathogenesis of Osteoarthritis. *Mediators of Inflammation*, 2014, pp.1–19.
- Xu, Y., Guo, Y., Liu, K., Liu, Z., & Wang, X., 2016. E-Cigarette Awareness, Use, and Harm Perception among Adults: A Meta-Analysis of Observational Studies. *Plos One*, 11(11), pp.e0165938.
- Zhang, G., Wang, Z., Zhang, K., Hou, R., Xing, C., Yu, Q., & Liu, E., 2018. Safety Assessment of Electronic Cigarettes and Their Relationship with Cardiovascular Disease. *International Journal of Environmental Research and Public Health*, 15(1), pp.75.