Analysis of Tumor Necrosis Factor-Alpha Levels and Nasal Mucociliary Clearance Time between "Biosmart and Safe Bus" and Regular Bus Passengers

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Abstract. Background: Buses are the most popular means of transportation and have the potential to spread the virus due to their high density in confined spaces, and poor air circulation. The application of the healthy triangle concept in the 'BIOSMART AND SAFE BUS' is an innovation by engineering the bus cabin environment in a 'smart' and 'safe' way. Exposure to pollutants and microorganism in bus passengers will trigger an inflammatory response by increase TNF- α levels and dysfunction of nasal mucociliary clearance. Therefore, further studies are needed on the examination of TNF-α levels and nasal mucociliary transport time in passengers on Biosmart and safe buses and regular buses.

Aim: Analyzing differences in TNF-α levels and Nasal Mucociliary Clearence time (NMC) between passengers of Biosmart and Safe and Regular Buses before and after the trip.

Method: Quantitative research using quasi-experimental research design with pre-test and post-test randomize control trial approach. Subjects were divided into two groups namely, the control group (regular bus passengers) and the treatment group (biosmart and safe bus passengers) (n = 86, each group n = 43). Both

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groups were examined for TNF-α level and nasal mucociliary clearance, before and after treatment.

Result: Based on the analysis, the mean value of pre-test in NMC time for control group was 14.88 ± 6.34, post-test 17.79 ± 5.99. Pre-test for treatment group was 13.79 ± 4.88 and post-test 15.51 ± 6.25. There was no significant difference between pre-test and post-test of NMC time between control and treatment group (P = 0.321). The mean value in TNF-α of control group pre-test was 26,63 ± 5,55 and post-test was 62,86 ± 5,28. The mean value of the pre-test in the treatment group was 25,53 ± 9,34 and the post-test was 62,45 ± 4,83. Data analysis of TNF-α pre, TNF- α post and TNF- α differences showed no significant difference between the treatment and control groups (P = 0,139, P = 0.708, P = 0.362).

Conclusion: There are statistically significant differences increase TNF-α levels of Biosmart and Safe Bus dan Regular Bus passengers after the trip. There is a statistically significant difference nasal mucociliary transport time of regular bus passengers after the trip and there is no significant difference nasal mucociliary transport time on the Biosmart and Safe Bus passengers after the trip.

Keywords: Biosmart and Safe Bus · TNF-α · nasal mucociliary clearance time (NMC)

1 Introduction

Bus is a means of land transportation that provide comfort and convenience for travelers in the community [1]. However, buses have characteristics that have the potential to spread the virus, namely the alternating flow of passengers in and out, high density in confined spaces, and poor air circulation. Inadequate ventilation and congested conditions in the bus micro environment can increase the risk of transmitting infectious diseases through the air [2]. Data on the spread of the SARS-CoV-2 coronavirus pandemic in Indonesia were reported since the first case appeared until October 2021, there were 4,242,532 confirmed cases with a total confirmed cases 143,333 deaths and an incidence rate of 0.08/100,000 population per week [3].

The impact of COVID-19 on changes in people's behavior and activity patterns causes a decrease in population movement or mobility. This condition provides opportunities and challenges in developing a land transportation system that is able to adapt to changes in population mobility [4]. The application of the healthy triangle concept in the 'BIOSMART AND SAFE BUS' is an innovation product that maintains a healthy bus cabin environment because the bus cabin is engineered 'smartly' in terms of 'physical distancing' (arranging passenger seats), regulating and filtering air circulation with Heppa Filter and UV-C irradiation, as well as the application of nanosilver on the entire surface of the bus cabin to reduce the number and density of viruses (viral load) in the bus cabin. The definition of a 'safe' bus means that passengers on the bus are required to also wear an Acchadana® herbal mask, so that they are safe from the possibility of being exposed to pollutants or diseases from other passengers [5].

The land transportation sector plays a role in producing pollutants that have an impact on health, including particulate matter (PM), ground-level ozone (O3), NO2,

carbon monoxide (CO) and volatile organic compounds (VOC) [6]. Pollutant compounds deposited in the alveolus, provoke an inflammatory response that causes the alveolus to activate macrophages and an acute inflammatory response, which triggers the production of biomarkers. Tumor necrosis factor alpha (TNF-α) is a major biomarker and cytokine involved in the inflammatory process in the lung [7].

Nasal mucociliary transport is the main defense mechanism of the respiratory system. The inhaled particles adhere to the nasal mucosa, which lines the nasal cavity in a single layer, and the efficient and coordinated activity of the cilia transports mucus towards the oropharynx. In this way, mucociliary clearance protects the respiratory system against inhaled particles and microorganisms. Ineffective ciliary activity can lead to acute or chronic diseases of the upper and lower respiratory tract [8].

Exposure to pollutants in bus passengers will trigger an inflammatory response by increasing levels of TNF-α and impaired nasal mucociliary clearance. Therefore, further studies are needed on the examination of TNF-α levels and the rate of nasal mucociliary transport time in passengers of Biosmart and safe buses and regular buses which are influenced by environmental conditions, humidity, age and travel time.

2 Methods and Materials

This study is a quantitative study, with a Quasi experimental pre and post test randomized control trial design by comparing two groups, namely the control group and the intervention group with inclusion criteria: the subject is willing to sign the informed consent, the subject is in good health, the subject is an UNDIP student who is involved as intern and member of Biosmart and safe bus matching fund training, and the subject has a covid-19 vaccine certificate. The exclusion criteria were that the subjects consumed alcohol during the study period, the subjects smoked during the study, the subjects were pregnant or menstruating and the subjects used inhalers during the study.

2.1 Research Sample

The total sample of 86 participants was obtained from the criteria, inclusion and exclusion. Participants were divided into two groups of passengers on Biosmart and Safe Bus ($n = 43$) and Regular Bus ($n = 43$) with randomized sampling. They were given and later filling out the screening questionnaire and Informed Consent. Participants were taken nasal wash samples for examination of TNF-α levels and examination of the rate of nasal mucociliary transport before and after the trip. Pre-test sampling (Nasal Wash) and administration of saccharin tablets were carried out by an ENT-KL specialist. Bio Smart and Safe Bus passenger participants were given Acchadana® herbal masks and regular bus passengers used medical masks. The mask that has been given is worn during the trip and replaced for 6–8 h or when it gets dirty. Participants boarded the bus according to the seats obtained randomly. Participants traveled for 21–25 h with a break of 9–14 h. Participants were taken post-test samples after the trip was finished.

2.2 Nasal Wash Sampling

Sampling was done using a nasal wash with the subject sitting position with the head extended 45°. Subjects were instructed to take a deep breath and hold their breath, a syringe containing 20 ml of distilled water was inserted into one nose, while the other nose was closed. The subject was then instructed to look down and slowly drain the fluid into the reservoir. The procedure is repeated in the other nostril. The procedure was carried out in two pre-test and post-test.

2.3 TNF-alpha

Analysis of TNF-α levels was obtained from nasal wash, nasal samples stored at 4 °C which were then examined using the Enzyme-Linked Immuno Sorbent Assay (ELISA) method based on the manufacturer's instructions. ELISA examination for nasal wash analysis of TNF alpha levels used the ELISA Kit96 wells from ABCLONAL. TNF-α concentrations were quantified after incubation by antibody detection. Analysis of TNF-α levels was carried out at the Laboratory Biomolecular of Muhammadiyah University, Semarang.

2.4 Measurement of the Nasal Mucociliary Transport Time Using the Saccharin Test

The process of saccharin tablets distribution started with the patient is checked under normal conditions and asked not to inhale, eat or drink, cough and sneeze. The subject was seated with the head flexed 10 degrees. Saccharin 10 mg tablets are placed 1 cm behind the anterior border of the inferior turbinate, then the patient is asked to swallow periodically for about -1 min until the patient feels the sweet taste. The time from when saccharin was placed under the inferior turbinate, the patient should press "ON" in the stopwatch until it tasted sweet and then he/she was instructed to turn off the stopwatch. The time was recorded and referred to as mucociliary transport time. In every 30 s, the patient was asked if there is a sweet taste. The participant's perceived time was recorded as mucociliary clearance time.

2.5 Statistic Analysis

All data were analyzed using SPSS, the 26 version. Data distribution of TNF-α and nasal mucociliary transport rate were tested using the Shapiro Wilk method. Primary data resulting from measurement of TNF-α and nasal mucociliary transport rate were analyzed using Mann-Withney, Wilcoxon, Kruskal Wallis, paired T and Independent T tests according to group type. Trend analysis is assessed by making a mathematical trend formula for delta/difference in TNF-α levels to see the trend in the direction of the graph.

Table 1. Description of Respondent Characteristics Data

Variable	F	%	Mean ± SD	Median (min – max)
Bus				
Regular	43	50,0		
Biosmart	43	50,0		
Age			20,66 ± 1,04	21 (18 – 23)
BMI			22,43 ± 3,66	21,8 (15,7 – 33,9)
Temperature			35,67 ± 0,75	35,8 (32,1 – 37,5)
Alcohol History	4	4,7		
Physical Activity	39	45,3		
Comorbid				
Respiratory Disorders	4	4,7		
Autoimmune History	0	0		
History of Liver, Lung, Heart	0	0		
History of Malignant Disease	0	0		
History of Hereditary Disease	0	0		
Pregnant/menstruation	0	0		
Smoking	10	11,6		
TMSH pre			14,34 ± 5,65	14 (5 – 29)
TNF-α pre			26,08 ± 7,66	25,86 (13,71 – 56,10)

Explanation: ‡ Mann whitney; £ Fisher's exact; ¥ Pearson chi square

2.6 Ethical Clearance

This study used a direct experimental test on humans. Therefore, before the research was carried out there was a submission of ethical clearance from KEPK FK Diponegoro University No: 412/EC/KEPK/FK-UNDIP/XI/2021 and Informed Consent from the bus passengers under study.

3 Research Results

Subjects were obtained from participants who passed the inclusion and exclusion criteria as many as 86 participants. Analysis of participant characteristics was assessed based on age, body mass index, temperature, alcohol history, health and smoking (Table 1).

3.1 Tumor Necrosis Factor Alfa (TNF-α)

The results of descriptive data TNF-α levels obtained that the normality of the data $p > 0.05$ was only found in the difference in the Regular Bus group. The mean value in the

Table 2. The difference between TNF-α pre test, post test and the gap

TNF-α	Bus		P
	Regular	Biosmart	
Pre test	26,63 ± 5,55	25,53 ± 9,34	0,139‡
Post test	62,86 ± 5,28	62,45 ± 4,83	0,708§
p	<0,001¶*	<0,001†*	
Gap	36,23 ± 8,19	36,92 ± 9,98	0,362‡

Explanation: * Significant (p < 0,05); § Independent t; ‡ Mann Whitney; Paired t; † Wilcoxon

Regular Bus group was 26,63 ± 5,55 for the pre test and 62,86 ± 5,28 for the post test value. Meanwhile, the Biosmart and Safe Bus group obtained an average value of 25,53 ± 9,34 for the pre test and 62,45 ± 4,83 for the post test value.

The results of Table 2 show that the results of the paired difference test on regular buses and biosmart and safe buses are significant or there are significant differences. In the unpaired difference test, it was found that the TNF-α pre, TNF-α post and the difference in TNF-α were not significant or there was no significant difference between the regular Bus and Biosmart and safe bus.

The results show (Table 2, Fig. 1) that the average difference in TNF- α levels in the regular bus group experienced an increase between the pre test and post test and the Biosmart and safe bus group which slightly increased. However, this mean difference shows that the results are not statistically significant with p value = 0.139 in the pre test and 0.708 for the post test (P < 0.05) in the Regular Bus or Biosmart and safe bus groups. From the pre-test and post-test scores, the Regular Bus group showed statistically significant difference test results with a p value =< 0.001 and in the Biosmart and safe bus group, which showed an insignificant difference test with a p value = 0.001. From the test results, the different values of the delta in the two groups showed an insignificant p value with a value of 0.362.

3.2 Nasal Mucociliary Clearance Time

The results of the NMC time descriptive data obtained that the normality of the data p > 0.05 was found in the difference between the two Bus groups. The mean value in the Regular Bus group was 14.88 ± 6.34 for the pre test and 17.79 ± 5.99 for the post test value. Meanwhile, the Biosmart and Safe Bus group obtained an average value of 13.79 ± 4.88 for the pre test and 15.51 ± 6.25 for the post test value.

The unpaired difference test on the regular bus is significant or there is a significant difference while on the Biosmart and Safe Bus it is not significant (Table 3). In the unpaired difference test, the NMC time pre, NMC time post and NMC time differences were not significant or there was no significant difference between the biosmart and regular (Table 3, Fig. 2).

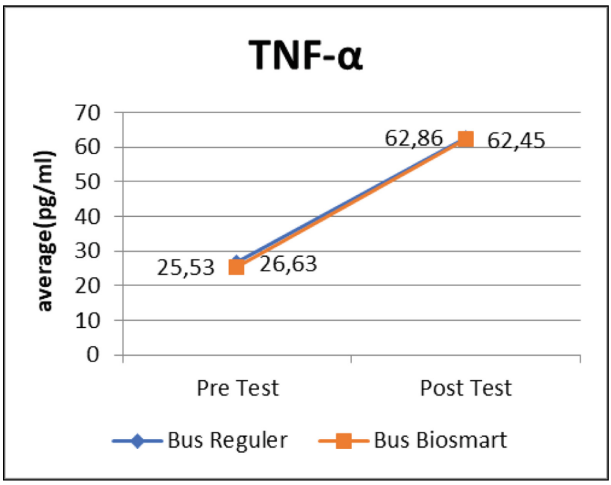

Fig. 1. Graph of the average Pre and Post TNF- α Levels between the Regular Bus Group and the Biosmart and Safe Bus

Table 3. The difference between NMC time pre test, post test and the gap

NMC Time	Bus		P
	Regular Mean ± SD	Biosmart Mean ± SD	
Pre test	14,88 ± 6,34	13,79 ± 4,88	0,582‡
Post test	17,79 ± 5,99	15,51 ± 6,25	0,051‡
p	0,001†*	0,085†	
Gap	2,91 ± 5,50	1,72 ± 5,52	0,321§

4 Discussion

4.1 Analysis of TNF- Levels in Passengers of Biosmart and Safe and Regular Buses

Based on the basic data of research subjects, TNF-α levels in the treatment group after the trip obtained an average increase of 62,45 ± 4,83 with p value =< 0.001; while the control group obtained an average increase of 62,86 ± 5,28 with p value =< 0.001 (Table 2). Increased levels of TNF-α after the trip is possible that there is a risk of an inflammatory response in the sinonasal which can cause clinical symptoms and signs in bus passengers. The results of research by Juliana J et al. stated that TNF-α is a biomarker of the respiratory system that plays a role in the respiratory inflammatory response. Where the pollutants caused by traffic pollution will trigger an inflammatory response according to the variation of the individual human being exposed to. Previous studies have shown that exposure to air pollutants ($PM_{2.5}$ PM_{10} and NO_2) significantly

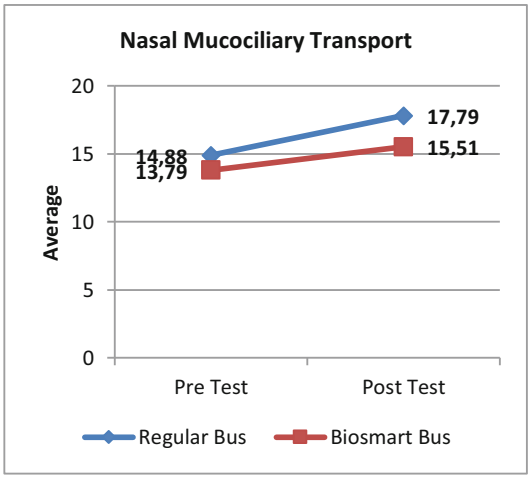

Fig. 2. Graph of Average Pre and Post NMC time between the Regular Bus and Biosmart and safe bus groups

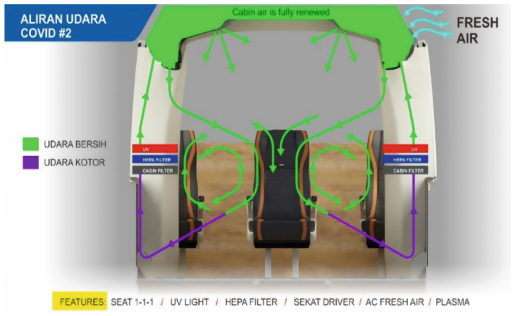

Fig. 3. Laminar flow pattern Biosmart and Safe Bus

affects the increase in TNF-α levels of sputum samples in school children [9]. Meanwhile, the results of research in Malaysia explained that the relationship between particulate matter and TNF-α levels in 62 samples of drivers bus as an exposed group and 62 samples of administrative staff as a comparison group showed TNF-α levels ($z = -5.88$, $p < 0.01$) were significantly higher in bus drivers [7]. Other research explains that Particulate Matter can induce oxidative stress and inflammation in nasal epithelial cells which cause upper respiratory tract infections [10].

Biosmart and safe buses have a healthy cabin eco-biological environment because they are engineered 'smartly', because of; a) in terms of 'physical distancing' (passenger seat arrangement), b) regulation and filtering of air circulation, and c) application of nano-silver on all surfaces of the bus cabin to reduce the number and density of viruses. The basis for regulating the airflow pattern of Biosmart and Safe Bus uses a laminar flow pattern, namely by adjusting the shape and size of the ducting, adjusting the position of

the air outlet and air inlet, as well as setting the capacity of the incoming air and outgoing air which aims to increase the supply of incoming clean air (Fig. 3) [11].

Unpaired difference test results obtained on TNF-α pre, TNF-α post and the difference in TNF-α was not significant or there was no significant difference between Biosmart and safe buses and regular buses. When the immune system decreases, the body will be more susceptible to disease. This is because the body's immune ability is weak to fight infection, causing TNF- to be produced in excess and TNF-α levels to increase. Age factors affect the strength or weakness of the immune system [12]. In this study, the age of the sample was still young, so they still had a good immune response and normal TNF-α levels. At a young age, hormone levels, especially growth hormone, are in optimal conditions. The function of growth hormone can suppress high levels of TNF-α. This has a good impact on TNF-α levels at a young age. Another factor that affects TNF-α levels is stress. When stress occurs, the glucocorticoid hormones and cortisol trigger an anti-inflammatory reaction of the immune system which causes an increase in TNF-α levels [13, 14].

The application of a high-efficiency particulate air (HEPA) filter before and after the fan is able to block particles up to 0.3 microns in size and UV-C ultraviolet irradiation effectively kills 99.9 percent of the viruses that cause COVID-19 in the air in 25 min [11]. The results of this study showed that TNF-α levels were significantly lower for passengers on Biosmart and safe buses than regular buses after the trip. TNF-α is a good biomarker of endotoxin response and increases after 90 min of stimulation. The normal concentration of TNF-α in serum is <35 pg/ml and becomes >240 pg/ml in sepsis and serum TNF-α levels in the blood will reach peak levels in the first 12 h after infection. The normal sputum TNF-α levels range from 10–100 pg/ml [13, 15].

The use of herbal masks on Biosmart and safe bus passengers can reduce the risk of exposure to pollutant compounds, pathogenic microorganisms and other compounds. In this study, the masks used during the trip were changed every 6–8 h to see their effectiveness [16]. Therefore, the protective power of the mask decreases if it is more than 10 h and is less effective in its function. This research is supported by previous research on the use of herbal masks and medical masks which have the ability to protect the airways of Biosmart and Safety Bus passengers as evidenced by increased levels of IgA and decreased levels of IL6. Meanwhile, the protective power is better using herbal masks but statistically almost close to significant [17, 18].

4.2 Analysis of the Nasal Mucociliary Transport Time on Biosmart and Safe Buses and Regular Bus Passengers

The results of the paired difference test showed that there was no significant nasal mucociliary transport time for Biosmart and safe bus passengers, and there was a significant difference for regular bus passengers. The mean post test results for nasal mucociliary transport time in the Biosmart and safe bus group were 15.51 ± 6.25 min and in the regular bus group was 17.79 ± 5.99 min. Based on these results, there is a difference in the average nasal mucociliary transport time after the trip in the Biosmart and safe bus group and the regular bus group which tends to be longer.

Mucociliary clearance function is a crucial defense mechanism of the nose and paranasal sinuses. Disruption of this function causes accumulation of secretions and

secondary infections. Temperature, humidity, pH and partial oxygen pressure are factors that affect this function [19]. Furthermore, It is necessary to evaluate the saccharin test because of the subjectivity of the research subjects and the possibility of differences in measurement methods on environmental factors, especially room temperature and humidity factors [20].

Based on the results of the unpaired different test of nasal mucociliary transport time in the Biosmart and safe bus group before and after the trip obtained a difference of 1.72 ± 5.52 min and the Regular Bus group before and after treatment obtained a difference of 2.91 ± 5.50 min with a value $p = 0.321$. The results of a nonsignificant time difference in nasal mucociliary transport time are consistent with the systematic review describes possible factors that can affect the rate of nasal mucociliary transport such as age, body temperature, drugs (such as adrenaline, acetylcholine, corticosteroids, and intranasal narcotics), tobacco use and smoking, and environmental factors (such as pollutants, smoke, and alcohol and dust) in addition to pathological conditions such as allergic rhinitis, acute or chronic rhinosinusitis, and nasal septal deviation [21].

Based on research on normal nasal mucociliary transport time was determined up to 20 min. The duration of 30 min is considered as the limit point that distinguishes normal subjects from subjects with impaired nasal mucociliary transport time [22]. The results of this study stated that the mean value in the Regular Bus group was 14.88 ± 6.34 for the pre test and 17.79 ± 5.99 for the post test value. Meanwhile, the Biosmart and Safe Bus group obtained an average value of 13.79 ± 4.88 for the pre test and 15.51 ± 6.25 for the post test value. Thus, the rate of nasal mucociliary transport in the subjects of this study was still within normal limits where traveling by bus was at risk of causing mucociliary disorders. On the Biosmart and safe bus passengers, the average results are shorter than the regular bus passengers after the trip, so it can be concluded that the Biosmart and safe Bus concept is able to maintain the mucociliary function of bus passengers.

The use of herbal masks and medical masks aims to protect bus passengers from exposure to infectious agents, including bacterial, fungal, and viral pathogens during the trip. During breathing through masks, the humidity, temperature, and resistance of inhaled air are altered [23]. In our study, a statistically significant prolongation was noted in mucociliary transport time (MCT) in the Regular Bus group with medical masks, whereas no statistically significant prolongation was observed in MCT in the the Biosmart and safe bus with Acchadana® herbal masks. This prolonged nasal mucociliary transport time detected in the regular bus group may be due to changes in temperature, humidity, pH of the breathing air or irritant rhinitis. In addition, some types of masks cause higher resistance to inhaled air, which results in negative pressure on the nasal mucosa [24].

In the literature, similar to our study Biosmart and safe bus passengers, that using the Accadhana herbal mask containing Nephrolepis exaltata – Hibiscus rosa sinensis in medical masks and then using it on motorcycle taxi drivers for 2 weeks can reduce IgA levels and IL-6 in sinonasal and increase levels of FVC, FEV1 and PEF [25]. The concept of Biosmart and safe Bus can be possible to maintain the mucociliary function of bus passengers.

There are weaknesses in this study, including environmental measurements of the levels of harmful compounds that can affect TNF- α levels and nasal mucociliary transport time in bus passengers.

5 Conclusion

The conclusion of this study is that there are statistically significant differences increase TNF-α levels of Biosmart and Safe Bus dan Regular Bus passengers after the trip. There is a statistically significant difference nasal mucociliary transport time of regular bus passengers after the trip and there is no difference nasal mucociliary transport time on the Biosmart and Safe Bus passengers after the trip. Statistical data shows that the results of the nasal mucociliary transport time for Biosmart and Safe Bus passengers and regular bus duration are still within normal limits.

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