INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). COVID-19 infections were identified in November 2019 in China, and on March 11th, 2020, WHO declared COVID-19 a pandemic. As of July 9th, 2020, WHO reported 545,481 deaths worldwide caused by COVID-19 with a Case Fatality Rate of 4.6%. Indonesia reported its first case on March 2nd, 2020, and it is increasing rapidly across Indonesia. As of July 9th, 2020, the Ministry of Health Indonesia reported 70,736 confirmed cases of COVID-19, with 3,417 deaths (CFR 4.8%).

Respiratory rehabilitation after COVID-19 infection is highly recommended to restore respiratory function, fitness, and quality of life. There is no difference between respiratory exercise by wearing and not wearing a mask, and monitoring the progress of therapy after COVID-19 infection is essential. One of the monitoring methods is using Heart Rate Variability (HRV). HRV analysis helps to see the role of the autonomic nervous system in the inflammatory reflex, where emphasis on parasympathetic activity is associated with disease pathogenesis and excessive inflammatory response. Lung function (FVC and FEV1) positively correlated with HRV in healthy adults, and reduced HRV was found in patients with COVID-19 infection.

Comprehensive rehabilitation program improves lung function in post-COVID-19 patients. Conventional exercises such as deep and diaphragmatic breathing or using tools such as incentive spirometry have increased HRV. Kulur et al. showed improvement in HRV after doing breathing exercises with diaphragmatic breathing for three months in patients with ischemic heart disease and diabetes mellitus. Another study by Ajudia et al. showed improvements in autonomic responses (basal heart rate, immediate maximum heart rate, steady state heart rate) after exercising with incentive spirometry for three months in healthy subjects.

The COVID-19 pandemic, which requires social distancing, has disrupted rehabilitation services. Based on the
Incentive spirometry is a pulmonary rehabilitation method that is easy to use, safe, inexpensive, can be done at home without supervision and is accompanied by a visual display as an indication to the patient that breath flow or volume has been achieved. There is no research data on the description of HRV in post-COVID-19 infection patients and the effect of giving breathing exercises using incentive spirometry in post-COVID-19 infection. This study aims to analyze the effect of incentive Spirometry exercise for four weeks on heart rate variability of post-treatment COVID-19 patients at Dr. Sutomo Hospital Surabaya.

METHOD

This research is experimental with a Randomized Pretest and Posttest Controlled Group Design, conducted at the Medical Rehabilitation Installation of Dr. Sutomo Hospital Surabaya. The research was carried out from October 2021 to October 2022. The research subjects were post-treatment COVID-19 patients who came to the Medical Rehabilitation Installation of RSUD dr. Sutomo Surabaya. The research sample was taken by consecutive sampling in which all research subjects who met the inclusion criteria were included in the study until the number of subjects was met.

The inclusion criteria of this study are: (1) Patients post-treatment for COVID-19 at Dr. Soetomo Hospital Surabaya is no more than three months old and has been declared hostile for COVID-19 based on PCR or antigen swab results and is still experiencing post-COVID-19 cardiopulmonary symptoms (shortness of breath, fatigue, chest pain, and cough), (2) Age over 18-59 years, (3) Can understand and follow verbal instructions properly good, MMSE score 24-30, (4) Hemodynamically stable (Systolic blood pressure ≥ 90 and ≤ 160 mmHg, mean arterial pressure (MAP) ≥ 65 and ≤ 120, heart rate ≥ 60 and ≤ 120 beats per minute, temperature 36.5 - 37.5°C, oxygen saturation > 95%), has received standard therapy according to the patient's condition, (5) Willing to participate in this study by signing the research consent form, and (6) able to operate a smartphone or have family members who can operate smartphones that have video call applications such as Whatsapp, Telegram, Zoom, Skype which enable direct two-way video communication. Exclusion criteria for this study included: (1) Having a history of lung disease (coughing up blood, asthma or chronic obstructive pulmonary disease (COPD), active pulmonary tuberculosis, history of spontaneous pneumothorax, traumatic pneumothorax that did not heal completely, lung cancer, pleural effusion, and history of smoking) which has been excluded through history, physical examination and chest X-ray results during treatment, (2) Suffering from severe cardiorespiratory disorders which are ruled out through history, physical examination and ECG including unstable angina, blood pressure > 160/100mmHg, Arrhythmia, Heart failure degrees 3 and 4 NYHA criteria, (3) Neurological disorders that cause weakness of the trunk muscles so that the patient cannot maintain an upright position, (4) Have complaints in the nose and mouth that interfere with the use of tools (acute sinusitis, upper respiratory tract infections, use of dental prostheses that may interfere with the use of incentive spirometry devices) that have been removed through the anaesthesia and physical examination, and (5) Visual and hearing impairments that can interfere with audiovisual communication which is enforced through a visual examination with a Snellen chart and a modified whisper test. Dropout criteria included: (1) Not doing the specified exercise program for more than three consecutive days during the 4-week exercise program, (2) Not following instructions during the predetermined exercise program, (3) Illness or other conditions it was not possible to follow the exercise protocol, so they had to stop the exercise program, (4) the subject declared his resignation, and (5) died.

Subjects were randomized into treatment and control groups and control groups using a lottery in a sealed envelope (single-blind). The treatment group received training using incentive spirometry, while the control group received diaphragmatic breathing exercises according to the exercises recommended by PERDOSRI and WHO in post-COVID-19 infection patients. Each subject carried out the exercises according to what was taught at home daily, as evidenced by filling in the exercise supervision card signed by the agreed witness. Both groups were given five times interventions with ten times repetitions, each with an interval of one hour between exercises every day for four weeks. At the end of the fourth week, the patient was asked to return for a heart rate variability examination. HRV examination was carried out using a pulse sensor with the Polar H10 brand placed on the chest in a sitting position for five minutes. The HRV parameters evaluated in this study were the Root Mean Square of Success Differences between normal heartbeats (RMSSD), Standard Deviation of N-N intervals (SDNN), and LF/HF ratio of Heart Rate Variability (HRV).

Statistical tests were carried out at the beginning and end of the study SPSS version 26. Before statistical analysis for each data, a normality test was performed on the primary characteristic data using the Shapiro-Wilk test, followed by a homogeneity test (independent sample T-test) for each variable. The normality test results show that the primary variable data in the treatment and control groups before and after treatment are typically distributed, so a parametric test is used.

RESULTS

The total subjects of this study were 20 people, divided into two groups (treatment and control), with ten people in each group. All research subjects in the treatment and control groups could complete the research until the end of the study. The average age of the research subjects in the treatment group was 48.10 ± 9.81 years, while in the control group, the average age was 44.30 ± 6.80 years. The average height in the treatment group was 164.00 ± 6.67 cm, and in the control group, 165.60 ± 7.76 cm. The average body weight 484
in the treatment group was 71.20 ± 12.49 kg, and in the control group, 75.10 ± 9.41 kg. The average body mass index (BMI) in the treatment group was 27.29 ± 4.54 kg/m², and in the control group, 27.90 ± 4.83 kg/m². The characteristic of the subject is shown in Table 1.

Table 2 shows the values of the RMSSD, SDNN, and LF/HF ratios in the treatment and control groups at baseline. There was no significant difference between the two groups in all the variables tested.

In the measurement of the effect size of the treatment group, the RMSSD parameter has a very small effect size (Cohen's D = 0.09); the SDNN parameter has a moderate effect size (Cohen's D = 0.51), the LF/HF ratio parameter has a moderate effect size (Cohen's D = 0.61) (Table 3).

In the measurement of the effect size of the control group, the RMSSD parameter has a moderate effect size (Cohen's D = 0.67); the SDNN parameter has a minimal effect size (Cohen's D = 0.12), the LF/HF ratio parameter has a minimal effect size (Cohen's D = 0.10) (Table 4).

There was no difference in the values of ΔRMSSD, Δ SDNN, and ΔLF/HF ratio between the control and treatment groups (p = 0.067; p = 0.706 and p=0.540) at the end of the study. In measuring the effect size of the treatment group, the parameter ΔRMSSD has a negligible effect size (Cohen's D = 0.40); the Δ SDNN parameter has a moderate effect size (Cohen's D = 0.51), the Δ LF/HF ratio parameter has a moderate effect size (Cohen's D = 0.51) (Table 5).

### DISCUSSION

The average age of the research subjects in the treatment group was 48.10 ± 9.81 years, while in the control group, the average age was 44.30 ± 6.80 years. Aging causes a decrease in the global measure of HRV and may reflect reduced responsiveness of autonomic activity to external environmental stimuli with age. These changes appear to be influenced by changes in basal metabolism, hormonal regulation, and neural activity in the elderly. Reardon and Malik found a decrease in HRV measures in subjects over 70 years of age.

In this study, in both groups, 80% of all study participants were obese. Several studies have shown an inverse relationship between weight gain and obesity with changes in HRV parameters. An increase in BMI can independently decrease HRV, especially when central adiposity is found.

In this study, HRV recording was carried out for 5 minutes (300 seconds), so the three most representative HRV domains were selected from the 5-minute measurement, namely RMSSD, SDNN,
and LF/HF ratio. At baseline, the average RMSSD and SDNN values in the two groups were below the normal range (RMSSD 19-75 ms and SDNN 32 – 93 ms), while the LF/HF ratio values were still within the normal range (LF/HF ratio 1.1 - 11.6). The study's result was similar to the study of Marquez et al., which showed lower HRV values in post-COVID-19 infection patients compared to normal controls. This study also showed a lower score in subjects undergoing treatment at the hospital. Kurtoglu et al. also showed that post-COVID-19 infection patients had decreased HRV values compared to ordinary people using a different HRV recording method for 24 hours.

HRV is a simple, objective, validated, non-invasive measure to assess autonomic nervous system function. High Frequency (HF), SDNN, and RMSSD describe the parasympathetic activity, while Low Frequency (LF) describes the sympathetic and parasympathetic activity. LF is the only indicator to assess sympathetic activity, so it is accepted as a parameter that describes it. The LF/HF index is considered to represent sympathetic and parasympathetic balance.

Several mechanisms can cause a decrease in HRV values in post-COVID-19 infection patients. COVID-19 infection can affect the work of the autonomic nerves through the cholinergic anti-inflammatory pathway, viral hijacking of the NF-KB transcription pathway, and autonomic balance. Like other diseases, the inflammatory process in COVID-19 infection will provide feedback to the central nervous system via afferent vagal signals to the Dorsal Vagal Complex (DVC). Feedback from the central nervous system will cause the release of acetylcholine (ACh) which will bind to α7-nicotinic-acetylcholine receptors (α7AchR's). This bond will stimulate monocytes to suppress the production of proinflammatory cytokines. This mechanism does not work correctly due to the COVID-19 infection, resulting in an imbalance in the autonomic response. This change in autonomic balance will become more evident as the COVID-19 infection worsen so that the HRV parameter can also be used as a reference for prognostic criteria in people with COVID-19 infection.

Another mechanism that can cause a decrease in HRV values in patients infected with COVID-19 is a decrease in lung function. COVID-19 infection impacts alveoli cell damage type 2, which will cause alveoli collapse, which can cause atelectasis. Decreased AT2 progenitor cells lead to impaired alveolar type I (AT1) cell regeneration, impairing alveolar repair and possibly increasing fibrosis. Both of these processes will cause lung recoil to decrease, increase airflow resistance in the lungs, increase the burden on the respiratory muscles and reduce respiratory volume.

Reduced lung volume and respiratory muscle weakness will result in decreased respiratory performance and oxygen exchange in the lungs, so oxygen pressure (PO2) decreases, and carbon dioxide pressure (PCO2) increases. Increased PCO2 can provide feedback to vagal receptors as well as negative feedback to baroreceptors which will affect the work of the sympathetic and parasympathetic nerves as seen in changes in heart rate, which will appear on HRV examination. Biachim et al. research showed that lung function (FVC and FEV1) positively correlated with HRV in healthy adults.

This study is the first to examine the effect of exercise using incentive spirometry on HRV changes in post-COVID-19 infection patients. Previous research by Ajudia et al. showed changes in autonomic responses, namely basal heart rate, immediate maximum heart rate, and steady-state heart rate, after doing exercise with incentive spirometry for three months in healthy subjects but using different parameters and more extended training time.

Incentive spirometry is often used in respiratory rehabilitation programs. Incentive spirometry is a device that measures the volume of air inhaled into the lungs during the inspiratory phase. Incentive spirometry uses visual feedback to assess the patient's inspiratory effort by measuring the inhalation volume, where the visual feedback encourages patient adherence to exercise.

Reychler study shows that Inspiratory Muscle Training and Incentive Spirometry help increase inspiratory muscle strength in elderly adults. In this study, incentive spirometry also provided additional benefits to increased chest mobility.

This study showed that giving incentive spirometry training to the treatment group for four weeks had a positive effect but did not significantly change the HRV parameters examined. The effect size calculation shows that the treatment group for four weeks can have a moderate effect on the SDNN parameter (Cohen’s D = 0.51) and the LF/HF ratio (Cohen’s D = 0.61). The absence of a significant effect on changes in HRV could be caused by several factors, including psychological factors that were not disclosed by research subjects during recruitment. A study by Khosari et al. showed that the prevalence of stress, anxiety, depression, and intrusion among health workers infected with COVID-19 was significantly higher than among health workers who were not infected. Research by Ahmed et al. also shows that most post-COVID-19 infection patients (91.2%) experience psychological problems for up to 6 months after infection. Several studies have shown a significant decrease in HRV values on RMSSD, SDNN, HF, and non-linear measures (MSE) in people with depression. HRV values also decrease in patients experiencing anxiety, including generalized anxiety disorder (GAD), social anxiety disorder, panic disorder, and post-traumatic stress disorder (PTSD).

The mechanism that causes a decrease in HRV due to psychological problems is still uncertain.

Another factor affecting the HRV assessment of the subjects studied is the level of physical activity after COVID-19 infection. Research conducted by Ahmed et al. showed that patients who survived post-COVID-19 infection found a

| Table 5. ΔRMSSD, Δ SDNN, and ΔLF/HF ratio in both group. |
|-----------------|----------------|-------|----------|----------|
|                 | Control Group  | Treatment Group | P value | Cohen’s D |
| Δ RMSSD (ms)    | 1.87 ± 2.75    | 0.40 ± 4.36     | 0.379    | 0.40      |
| Δ SDNN (ms)     | 0.87 ± 7.52    | 5.70 ± 11.07    | 0.269    | 0.51      |
| Δ LF/HF (ms²)   | -0.11 ± 1.06   | -0.65 ± 1.06    | 0.272    | 0.51      |
decrease in functional capacity, shown by a decrease in the 6 minutes walking test with slight improvement for six months after infection. The research by Tebar et al. showed a positive correlation between the HRV RMSSD and SDNN values in people who regularly engage in sports and a positive correlation in SDNN values in people who are actively carrying out occupational activities. The same result was also shown by a study conducted by Dietrich et al. showed higher SDNN values in both normal-weight and obese people who exercise regularly two times a week compared to sedentary and obese people.

The mechanism of increased physical activity towards improvements in HRV values is thought to be through neurohormonal improvements in more active people. Another mechanism is that physical activity can directly affect cardiomyocytes by increasing the strength of contraction and the stability of the flow of electricity in the heart. The sample in this study were patients with moderate and severe degrees of COVID-19 with an average length of stay of more than two weeks, so it is possible that some samples still have psychological problems, and their low activity level can affect their HRV measurement results.

In the control group, there was no increase in the RMSSD, SDNN, and LF/HF Ratio values. The effect size value shows that the diaphragmatic breathing program in the control group for four weeks was able to have a moderate effect on the RMSSD parameter (Cohen's D = 0.67) and had a minimal effect on the SDNN parameter (Cohen's D = 0.12) and LF/HF ratio (Cohen's D = 0.10). The effect of diaphragmatic breathing on HRV is primarily due to changes in the balance between sympathetic and parasympathetic nervous system activity in the heart. Diaphragmatic breathing is considered the healthiest form and one of the most straightforward yet powerful stress management techniques. Abdominal breathing evokes internal calm, relaxation, and peripheral warming, and long-term diaphragmatic breathing exercises lead to stable cardiac autonomic control modification and improve HRV.

This study results from the study of Alaguveni and Devaki, who examined the effects of diaphragmatic breathing in healthy young adult subjects for six months. Kulur et al. investigated the effect of twice daily diaphragmatic breathing exercises in ischemic heart disease patients with diabetes. This study showed an improvement in HRV after practicing diaphragmatic breathing for three months, and this increase will become even more significant after one year of practice. This difference is due to the shorter duration of the exercise in this study. Further research with a longer duration is needed to confirm this opinion.

The results of this study indicate that the average value of Δ SDNN and Δ LF/HF ratio in the treatment group is higher than that of the control group, with a moderate effect size (Cohen's D = 0.51; Cohen's D = 0.51), but there is no significant difference between the treatment and control groups. These results indicate that the incentive spirometry exercise significantly improves the Δ SDNN and Δ LF/HF ratio variables compared to the diaphragmatic breathing exercise.

Respiration is one of the most potent modulators of HRV through a mechanism known as Respiratory Sinus Arrhythmia (RSA). SDNN values and LF/HF ratios are closely related to baroreflex stimulus, which is mediated by RSA. Better improvement in the treatment group could be due to increased respiratory function after exercise using incentive spirometry. Biachim et al. research shows that lung function (FVC and FEV1) positively correlates with HRV in healthy adults.

The use of flow-oriented incentive spirometry shows better results in improving cardiopulmonary and functional parameters compared to diaphragmatic breathing exercises and volume-oriented incentive spirometry in several studies. Suharti et al. research in patients with COVID-19 showed functional improvement (4-meter gait time test and 30 sit-to-stand test) after five days of incentive spirometry exercises compared to 5 days of diaphragmatic breathing exercises. Shetty et al. in subacute stroke patients showed better lung function improvement and maximal respiratory pressures by giving flow-oriented incentive spirometry compared to giving diaphragmatic breathing exercises and volume-oriented incentive spirometry for five days.

The results of this study showed that the average value of Δ RMSSD in the control group was higher in the treatment group, with a small effect size (Cohen's D = 0.41). However, there was no significant difference between the treatment and control groups. These results indicate that diaphragmatic breathing exercises have a negligible effect on improving the ΔRMSSD variable compared to incentive spirometry exercises.

The RMSSD reflects beat-to-beat variation in HR, is less affected by respiratory sinus arrhythmia (RSA) than other time domain parameters, and is used to estimate parasympathetic system-mediated changes in HRV. The better effect on RMSSD in the control group compared to the treatment could be the relaxation effect, which can reduce stress. Stress will increase sympathetic activity and inhibit parasympathetic activity, characterized by a decrease in HRV parameters. Available evidence suggests that diaphragmatic breathing reduces stress as measured by physiological biomarkers and psychological self-report tools.

Diaphragmatic breathing exercises can reduce stress levels and increase HRV parameters, as proven in a study by Steffen et al., but this needs to be confirmed in further studies.

The limitations of this study are: (1) This study does not control matters affecting HRV recording (observation of sleep patterns, psychology, eating patterns, and physical activity of each outside treatment), (2) Distribution sample size based on BMI, gender, and the severity of COVID-19 is not evenly distributed, so an effect analysis cannot be carried out based on this grouping, (3) No research group was given a combination of exercises with incentive spirometry and diaphragmatic breathing.

CONCLUSION

For four weeks breathing exercises using incentive spirometry or diaphragmatic breathing did not significantly improve heart rate variability in post-COVID-19 infection patients. There is no difference between giving breathing exercises using...
diaphragmatic breathing for four weeks and breathing exercises using incentive spirometry for four weeks on heart rate variability in post-COVID-19 infection patients.

RESEARCH ETHICS
This research has been approved by the Research Ethics Committee, Faculty of Medicine, Universitas Airlangga, with ethical clearance number 0277/KEPK/X/2022.

CONFLICT OF INTEREST
There is no conflict of interest in writing this research report.

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AUTHOR CONTRIBUTION
All authors have made the same contribution in writing the report on this study result, from the proposal preparation stage, data search, and data analysis, to the interpretation of research data and presentation of the final report.

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