

Alteration in salivary cortisol and interleukin-6 levels during two different intensities of acute aerobic exercise

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Abstract

Problem statement: An increase in exercise intensity enhances the immune system. However, concentrations of cortisol and cytokine responses as acute effects of different exercise intensities need to be comprehensively elucidated. The purpose of this study was to examine the effects of different exercise intensities on concentrations of salivary cortisol and interleukin-6, a proinflammatory cytokine, at four distinct time points. **Research Methods:** Forty-five participants were classified into three groups (15 participants/group): one control group and two groups undergoing aerobic exercise at 65-80% maximum heart rate and >80% maximum heart rate, respectively. Saliva samples were collected from each participant at 0, 45, 60, and 90 min to measure cortisol and interleukin-6 levels using enzyme-linked immunosorbent assay kits. **Results:** Salivary cortisol levels at 60 min after exercise significantly differed between the aerobic exercise at 65-80% maximum heart rate and control groups ($P = 0.024$). Compared with the control group, aerobic exercise in the 65-80% and >80% maximum heart rate showed significantly different effects on altered salivary interleukin-6 levels at 45 and 60 min after exercise ($P = 0.004$ and $P = 0.016$, respectively). At 60 min, the concentration of salivary interleukin-6 was significantly higher in the aerobic exercise at 65-80% maximum heart rate than in the >80% maximum heart rate group ($P = 0.049$). **Conclusion:** Our findings revealed that different intensities of acute aerobic exercise might induce high levels of salivary cortisol and interleukin 6. It would be a beneficial tool for healthy individuals as they strive to rate the work intensity of aerobic exercise.

Keywords: aerobic exercise, interleukin-6, cortisol, saliva

Introduction

Exercise can improve the body's functional capacity, physiological systems, and mental health. Several studies have suggested that exercise can play an essential role in preventing multiple risk factors, such as certain cancers [1] while reducing the risk of heart disease, hypertension, osteoporosis, obesity, type II diabetes, high blood pressure, cholesterol imbalance and lipid profile [2,3,4]. However, exercise can cause systemic alterations in various hormones, including stress hormones [5] and responses to inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor- α (TNF- α) [6]. Cytokines are produced from several sources to regulate and influence the modulation of immune and inflammatory responses [7]. For example, excess lipids promote macrophage recruitment to eliminate bacteria, viruses, and substances via phagocytosis. In turn, the activation of immune cells in response to cytokines upregulates the inflammatory process by releasing proinflammatory cytokines [8]. IL-6 and TNF- α are two major proinflammatory cytokines secreted in notable amounts from adipose tissue, which is associated with higher levels of proinflammatory mediators [9-10]. IL-6 is among the most commonly assayed markers associated with an inflammatory response. An increase in IL-6 levels is reportedly the earliest and most prominent cytokine response to exercise, although it is strongly related to the intensity and duration of exercise [11]. Cortisol is a stress hormone produced by the adrenal glands [12] and plays a major role in metabolism and immune function during systemic alteration and psychological stress [13-14]. Previous studies have revealed that the cortisol concentration can be assessed at different time points during the circadian rhythm, as well as at different exercise intensities [15-16]. To determine the response to exercise, one study has evaluated exercise performed at intensities of 60% and 80% of maximal oxygen uptake (VO_{2max}) to determine the intensity at which an increase in circulating cortisol would be observed. The authors reported that low-intensity exercise (40% VO_{2max}) did not significantly increase cortisol levels [17]. Additionally, the cortisol response can vary depending on the type and intensity of exercise, level of training, nutritional status, and stress level during exercise [17-18]. According to a study by Budde et al., the athlete group showed a significant increase in salivary cortisol concentrations after competition performance when compared with that observed prior to performance [19]. However, the cortisol response to different intensities and durations of

exercise reportedly increases in response to long-duration high-intensity exercise [20-23]. As a result, increasing anxiety during exercise resulted in higher cortisol levels than trials with the same intensity but no emotional stress [24]. Furthermore, it is interesting to consider changed salivary IL-6 for a consequence of acute psychosocial stress and its biological correlates. Knowing the molecular correlates of salivary IL-6 in connection to psychosocial stress could help researchers better understand the underlying process at work. Although several studies have examined cortisol levels and cytokines [25-26], there is little knowing about the relationship between cortisol and IL-6 after acute aerobic exercise. In particular, the cortisol response to low-intensity and short-duration exercise remains unclear and warrants further investigation. Accordingly, in the present study, we aimed to investigate the effects of two different intensities of acute aerobic exercise on the modulation of salivary cortisol and IL-6 responses to acute exercise in healthy subjects.

Materials and Methods

Participants and study design

Forty-five healthy adults (17 men and 28 women) were recruited in the present study. The participants were young adults aged 18-35 years from the Tha Sala District, Nakhon Si Thammarat Province, Southern Thailand. The calculated sample size was 15 in each group using G*Power [27], at a significance level of 0.05 and power of 0.90, based on results obtained from previous studies [28]. All participants completed a questionnaire for demographic data, exercise history, and medical history, which were recorded for health screening purposes. All participants received a detailed description of the experimental procedure and provided written informed consent. Inclusion and exclusion criteria were as follows:

Inclusion criteria:

Participants performed all basic and instrumental activities of daily living under normal conditions and in the absence of ongoing depression and adequate social conditions.

Exclusion criteria:

Participants who were unable to exercise safely, had generalized anxiety disorder, experienced changes in medication or therapy in the past three months, acute illness or injury during exercise, abuse of alcohol or other drugs, and smoking. Participants were instructed not to self-medicate and to contact the physician in charge of the study in case of fever, acute infection, use of new medications. In addition, participants were asked to avoid altering their dietary habits.

Body composition

Body composition, including height, body weight (BW), percent fat (%fat), body mass index (BMI), bone mass, muscle mass, and basal metabolic rate, was measured before the start of the exercise session using a body composition monitor (Tanita model UM-076), as described in the manufacturer's guidelines.

Exercise conformation The exercise session was performed during the afternoon (14.00-16.00 am), as described in a previous study [28-29], at the same time and under similar environmental conditions, at the Physiology Laboratory, the Center for Scientific and Technological Equipment, Walailak University. The participants estimated their maximum aerobic capacity using a recumbent ergometer test. After subjects were classified in terms of physical conditioning, their working maximum heart rate (HRmax) was determined using the following formula: $208 - (0.7 \times \text{age})$ [30]. Eligible participants were further classified into three groups, who then performed a 10-min warm-up, a 5-min warm-up at a workload eliciting 40% of their maximum aerobic capacity, followed by exercise at 60-80% HRmax for 30 min, and >80% HRmax for 20 min, which maintained a constant heart rate using a polar heart rate monitor, according to the manufacturer's instructions. After the exercise session, participants completed a 30 min recovery session. In the control group, the participants did not engage in any type of exercise for at least 24 h, maintaining their normal daily activities throughout the study period. The design of the experimental setup is shown in Fig. 1.

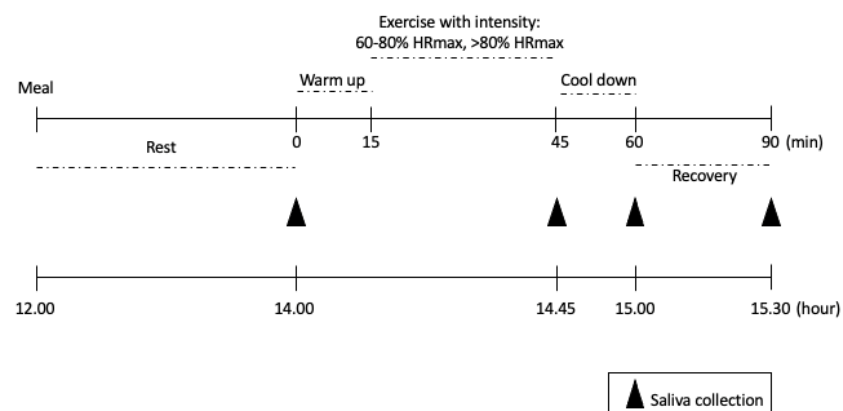


Fig. 1 The outline of the time schedule of the study.

Saliva collection procedure and storage

Participants were required to abstain from food or drinks and avoid performing any oral hygiene procedures for at least 2 h prior to saliva collection. Participants were then instructed to rinse their mouth with water thoroughly and wait for at least 10 min before saliva collection. Saliva samples were collected before, during, and after exercise at four distinct time points (0, 45, 60, and 90 min). The saliva flow rate was represented as the volume of the whole saliva (mL), which was estimated by weighing each sample to the nearest milligram. The nearest milligram of saliva was carefully collected in a clean and sterile container *and immediately stored at -80°C* until analysis. The saliva samples were thawed and centrifuged at $10,000 \times g$ for 10 min at 4°C to remove insoluble material and cellular debris. Supernatants were collected and used for all assays.

Detection of salivary cortisol

Salivary cortisol levels (25 µL) were measured using a competitive immunoassay kit (Salimetrics, PA, USA), according to the manufacturer's instructions.

Detection of salivary interleukin-6

Salivary IL-6 levels (100 µL) were measured using a sandwich ELISA kit (Abbkine, Inc., Wuhan, China), according to the manufacturer's instructions.

Statistical analyses

Data values are presented as the mean $\pm$ standard error of the mean (SEM). IBM SPSS Statistics 26 (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Paired sample t-tests were used for comparisons between groups. One-way analysis of variance (ANOVA) with Tukey's post-hoc test was performed to evaluate significant differences between the three groups. Statistical significance was set at $P < 0.05$.

Results

Demographic data of participants

Tab. 1 summarizes the demographic data of participants enrolled in the present study. The results demonstrated that heart rate was significantly higher in the aerobic exercise with 60-80% HRmax and >80% HRmax group than control ($P = 0.0001$).

Characteristic	Group (Mean $\pm$ SEM)		
	Control (n = 15)	Aerobic exercise	
		60-80% HRmax (n = 15)	>80% HRmax (n = 15)
Age (year)	23.40 $\pm$ 1.77	23.80 $\pm$ 1.82	22.33 $\pm$ 1.61
Height (cm)	162.20 $\pm$ 1.25	162.00 $\pm$ 1.74	169.80 $\pm$ 2.15*,##
Weight (kg)	61.05 $\pm$ 2.82	56.15 $\pm$ 3.16	66.35 $\pm$ 2.77#
BMI (kg/m ²)	23.04 $\pm$ 1.24	20.71 $\pm$ 0.92	23.51 $\pm$ 0.86
%Fat (%)	27.87 $\pm$ 3.09	22.05 $\pm$ 1.72	23.14 $\pm$ 2.58
Muscle mass (kg)	41.50 $\pm$ 0.69	41.33 $\pm$ 2.44	47.04 $\pm$ 2.26
Bone mass (kg)	2.47 $\pm$ 0.17	2.38 $\pm$ 0.12	2.84 $\pm$ 0.13
Basal metabolic rate (kcal)	1343.29 $\pm$ 20.23	1291.13 $\pm$ 60.62	1503.34 $\pm$ 61.51*
Heart rate (bpm)	62.73 $\pm$ 1.43	137.67 $\pm$ 2.34****	165.13 $\pm$ 1.33****,###

Tab. 1 Data are presented as mean $\pm$ standard error of the mean (SEM). * $P < 0.05$ indicates a significant difference when compared with the control group. # $P < 0.05$, ## $P < 0.01$ indicates significant difference between 60-80% HRmax and >80% HRmax. **** $P < 0.0001$ indicates a significant difference when compared with the control group. #### $P < 0.05$ indicates a significant difference between 60-80% HRmax and >80% HRmax group, with one-way ANOVA (Tukey's multiple comparison test). BMI, body mass index.

Salivary cortisol responses during exercise

Tab. 2 summarizes the mean salivary cortisol level, we observed that the mean salivary cortisol level was unaltered in the 60-80% HRmax group and >80% HRmax group ($P = 0.282$ and 0.231 , respectively). The mean salivary cortisol concentration in the aerobic exercise with 60-80% HRmax group was significantly increased at 60 min after exercise ($P = 0.024$) when compared with the control group. Considering, we observed that the mean salivary cortisol concentration in the aerobic exercise with 60-80% HRmax group was significantly higher at 60 min after exercise than before exercise initiation ($P = 0.0434$), as shown in Fig. 2. Furthermore, the mean

salivary cortisol concentration in the >80% HRmax group did not significantly differ at 45, 60, and 90 min when compared with that recorded before exercise initiation. In addition, cortisol levels did not significantly differ between men and women participants at any examined time point, as shown in Fig. 4.

Time (min)	Group	Intensity	P-value	95% CI	
				Lower	Upper
0	Control	60-80% HRmax	0.977	-0.156	0.132
		>80% HRmax	0.248	-0.239	0.478
	60-80% HRmax	>80% HRmax	0.341	-0.227	0.059
45	Control	60-80% HRmax	0.282	-0.258	0.058
		>80% HRmax	0.231	-0.266	0.049
	60-80% HRmax	>80% HRmax	0.992	-0.166	0.15
60	Control	60-80% HRmax	0.024*	-0.721	-0.043
		>80% HRmax	0.679	-0.457	0.222
	60-80% HRmax	>80% HRmax	0.153	-0.749	0.603
90	Control	60-80% HRmax	0.19	-0.446	0.069
		>80% HRmax	0.251	-0.439	0.769
	60-80% HRmax	>80% HRmax	0.997	-0.25	0.265

Tab. 2 Salivary cortisol concentrations in samples were measured at four time points using ELISA. One-way ANOVA with Tukey's multiple comparison test was used to compare the data to the control group. * $P < 0.05$.

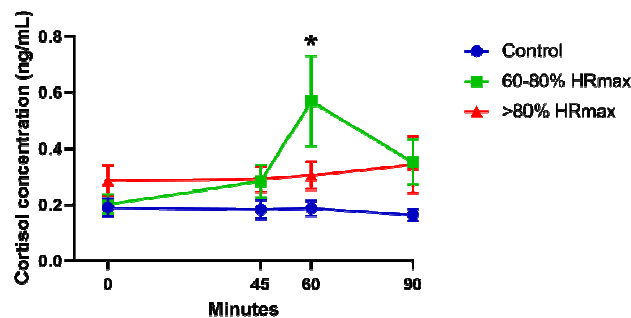


Fig. 2 Salivary cortisol concentration of three group, as determined by ELISA. Data are presented as mean $\pm$ standard error of the mean (SEM). * $P < 0.05$ indicates a significant difference when compared with the control group, with ANOVA (Tukey multiple comparison test). HRmax, maximum heart rate; CI, confidence interval

Salivary IL-6 responses during acute exercise

Salivary IL-6 levels are shown in Tab. 3. At 45 and 60 min after exercise, the mean salivary IL-6 concentration was significantly higher in the aerobic exercise with 60-80% HRmax group than in the control group ($P = 0.004$ and $P = 0.016$, respectively). At 90 min post-exercise, the mean salivary IL-6 concentration in the aerobic exercise >80% HRmax group was significantly higher than that before exercise initiation. Moreover, salivary IL-6 levels were higher in the aerobic exercise with >80% HRmax group than in the aerobic exercise with 60-80% HRmax group at all time points examined; however, the observed differences were not statistically significant, as shown in Fig. 3. In addition, IL-6 levels did not significantly differ between men and women participants at any time point, as shown in Fig. 4.

Time	Group	Group	P-value	95% CI	
				Lower	Upper
0	Control	60-80% HRmax	0.802	-0.322	0.188
		>80% HRmax	0.895	-0.208	0.303
	60-80% HRmax	>80% HRmax	0.529	-0.141	0.369
45	Control	60-80% HRmax	0.004*	-0.779	-0.127
		>80% HRmax	0.621	-0.451	0.201
	60-80% HRmax	>80% HRmax	0.049 [#]	0.001	0.653

60	Control	60-80% HRmax	0.016*	-0.749	-0.067
		>80% HRmax	0.156	-0.606	0.077
90	60-80% HRmax	>80% HRmax	0.567	-0.198	0.484
	Control	60-80% HRmax	0.078	-0.76	0.032
		>80% HRmax	0.132	-0.718	0.075
	60-80% HRmax	>80% HRmax	0.963	-0.354	0.439

Tab. 3 Interleukin-6 concentrations were measured in samples at four time points using ELISA. Data were compared with the control group using one-way ANOVA with Tukey's multiple comparison test. * $P < 0.05$ indicates a significant difference when compared with the control group. # $P < 0.05$ indicates a significant difference when compared with 60-80% HRmax and >80% HRmax group.

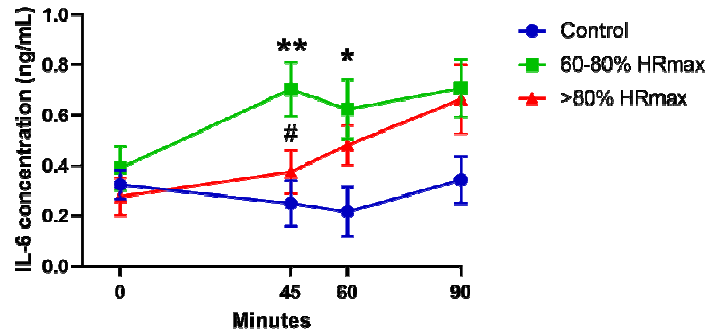


Fig. 3 Data are presented the IL-6 levels as mean $\pm$ standard error of the mean (SEM). * $P < 0.05$; ** $P < 0.01$ indicates significant difference when compared with the control group. # $P < 0.05$ indicates a significant difference when compared with the 60-80% HRmax and >80% HRmax groups, with ANOVA (Tukey multiple comparison test).

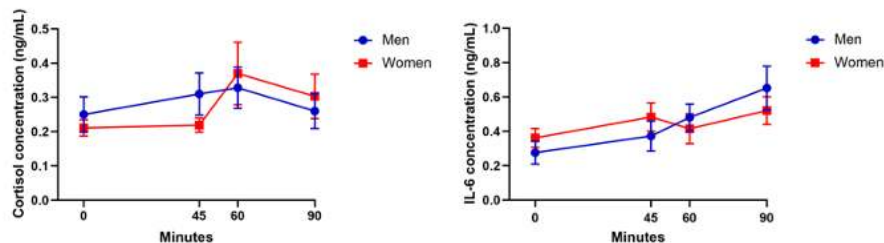


Fig. 4 Comparison of salivary cortisol and interleukin-6 concentrations in men and women participants. (A) Cortisol concentration in men and women. (B) Interleukin-6 concentration in men and women. Data were compared between men and women participants using an unpaired t-test.

Discussion

Herein, we aimed to examine the concentration of salivary cortisol and IL-6 during acute aerobic exercise performed at two distinct intensities (60-80% HRmax and >80% HRmax) and measured at different time points (0, 45, 60, and 90 min), given that cortisol is commonly measured in the plasma and serum during exercise [31]. The main finding revealed that the aerobic exercise groups with 60-80% HRmax and >80% HRmax showed markedly different responses in terms of both cortisol and IL-6 concentrations. Studies have previously reported elevated salivary cortisol concentrations post-exercise [32-36]. However, our study is the first to demonstrate that the salivary cortisol concentration was significantly increased immediately after 60 min of aerobic exercise in the 60-80% HRmax group, consistent with a previous report [16]. Although cortisol concentrations were affected by exercise performed at different intensities, they were recorded post-exercise for 1 h [34], as cortisol concentrations remained unchanged immediately after exercise. However, the previous investigation failed to observe any significant increase in cortisol levels at any intensity up to 40 min post-exercise. It can be postulated that changes in cortisol concentrations are in response to exercise but are not observed immediately after exercise. Another study has demonstrated that the autonomic nervous system regulates changes in the composition of salivary secretion via sympathetic stimulation [35]. Herein, we observed an increased salivary cortisol concentration under aerobic exercise at two intensities (60-80% HRmax and >80% HRmax), revealing that high salivary cortisol responses show a decreasing trend after aerobic exercise. Accordingly, it is plausible that the duration and/or intensity of exercise performed in the present study was

insufficient to achieve this threshold. Moderate-to-high-intensity (60% to 80%) exercise will provoke a significant increase in circulating cortisol levels by enhancing secretory stimuli within the hypothalamic-pituitary-adrenal axis; however, low-intensity exercise does not significantly increase circulating cortisol levels [16]. It should be noted that the high intensity of exercise could be attributed to several factors [37], as highly trained individuals typically have a higher intensity threshold necessary to provoke an increase in cortisol [34].

Along with changes in salivary cortisol levels, salivary levels of inflammatory cytokine were also elevated. Aerobic exercise with 60-80% HRmax could increase IL-6 levels in circulating saliva after 45 and 60 min of exercise. However, the exercise-induced increase in IL-6 concentration was not linear over time, and repeated measurements during exercise showed an almost exponential increase in plasma IL-6 [38-39]. As an example of IL-6 response to acute exercise (60-80% HRmax), the concentration of IL-6 peaked during acute exercise, followed by a slight decrease at the end of acute exercise. Thereafter, the increase in IL-6 level was gradual and remained elevated after acute exercise, higher than basal levels. In addition, acute exercise with >80% HRmax intensity gradually increased the IL-6 concentration. Increased IL-6 levels have been reported using different exercise intensities and duration [40-42] and can be associated with muscle capacity or hypertrophy to mobilize energy [43]. Importantly, the contracting skeletal muscle releases IL-6 into circulation in response to exercise. IL-6 mRNA content is extremely low in resting human skeletal muscle, while small amounts of IL-6 protein could be detected using sensitive immunohistochemical methods, predominantly in type I fibers [44]. In response to exercise, an increase in the IL-6 mRNA content in the contracting skeletal muscle is detectable after 30 min of exercise, and up to a 100-fold increase in the IL-6 mRNA content could be detected at the end of the exercise period [45]. However, the combination of mode, intensity, and duration of exercise can affect the magnitude of the exercise-induced increase in plasma IL-6 concentration. In the present study, IL-6 might act locally within the contracting muscle during exercise or within the adipose tissue during recovery, while most other cells and target organs are exposed only to IL-6 released into the systemic circulation. Although systemic factors may modulate the response, local factors appear to be crucial. Regarding the systemic effects of IL-6, the dose-response relationship and timing must be considered.

IL-6 is likely to play a central role in the cytokine cascade as a proinflammatory cytokine, mainly because IL-6 levels are elevated prior to other cytokines. As expected, the IL-6 induced increase in salivary cortisol was in agreement with previous findings [46], suggesting that increased circulating neutrophils can be attributed to elevated plasma cortisol [26]. However, our study did not observe circulating neutrophils in the plasma during exercise. Although a modest increase in plasma IL-6 can induce cortisol, marginally higher plasma IL-6 concentrations appear necessary to consistently increase plasma glucagon and growth hormone levels [47]. Hence, aerobic exercise-induced elevated IL-6 levels stimulated increased cortisol levels. Overall, the results of this study are useful tool to investigate the role of exercise for healthy and training volume during aerobic exercise on both IL-6 and salivary hormonal responses.

One limitation of the present study was that cortisol and IL-6 concentrations were measured during a short period of acute exercise. Moreover, we analyzed salivary levels of cortisol and IL-6. To examine cortisol levels in healthy individuals and athletes, blood sampling is the most frequently recommended method to identify differences in cortisol levels during each exercise phase. In addition, the sample size for each group was relatively small, and we considered the intensity of exercise, specifically anaerobic exercise, in this study. Future studies should compare the responses of cortisol and IL-6 in blood samples and examine stress markers and IL-6 in various types of athletes while precisely determining the time point at which blood samples should be collected. Moreover, the modulations of the cytokine responses and inflammatory reactions and their recovery need further study.

Conclusions

In the present study, our findings revealed that salivary cortisol and IL-6 concentrations are differently changed during acute aerobic exercise. The levels of cortisol and IL-6 may be affected by differences in intensity and effort. As a result, our findings support the practice of obtaining immunological effects in the acute impacts of various exercise intensities. It would be a useful tool for healthy people who want to rate the work intensity of aerobic exercise and also aerobic exercise training in order to lower the chance of developing a health problem. To acquire a better knowledge of the relationship between acute aerobic exercise and their genuine influence on immunological markers, future studies should address both external and internal factors.

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Author contribution statement

All authors: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, and validation. SH: writing the original draft of manuscript. CP and LL: review and revision. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published Article.

Declarations

The study was conducted according to the declaration of Helsinki and was approved by the Human Research Ethics Committee of Walailak University (approval number: WUEC-21-023-01). The study protocol was fully explained to all participants, and written informed consent was obtained prior to enrollment.

Consent for publication - Not applicable.

Competing interests - The authors declare that they have no competing interests.

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