Research proposal

1. Project title

The effects of different inspiratory pressures on the diaphragmatic thickness fraction and sternocleidomastoid muscle activation in people after stroke

2. Abstract

This study is a cross-sectional study to determine the optimal inspiratory muscle training (IMT) intensity for stroke survivors. Participants will breathe through a pressure threshold inspiratory loading device with varying loads in random order. During the test, accessory inspiratory muscle (sternocleidomastoid muscles) activity will be measured with surface electromyography (sEMG) and diaphragm thickness will be used to assessed with ultrasonography. Repeated-measures ANOVA will be used for statistical analysis to determine the most effective training intensity.

3. Project objectives

Introduction:

Stroke was the second largest cause of global death and disability in 2019 [1]. Direct medical costs for stroke in China was 50 billion yuan (nearly 7.69 billion US dollars) in 2015, and this accounted for the highest medical cost in China [2]. In 2016, the Global Burden of Disease (GBD) study estimated that the lifetime risk of stroke from the age of 25 years was 39.3% in China, which is the highest estimated risk in the world [3]. In 2018, stroke was the third highest disease cause of death, accounting for 1.57 million deaths in China [4].

Stroke not only causes impairment of motor function but can also affect lung function [5]. The lung function (e.g. forced vital capacity (FVC), forced expiratory volume in one second (FEV1), maximal inspiratory pressure (MIP), peak expiratory flow (PEF)) in people after stroke was reported significantly lower than the age and gender-matched healthy adults [6, 7]. Impaired lung function in people after stroke not only leads to high risk of pneumonia, poor functional outcomes and prolonged hospitalization but may also increase mortality [8-10].

Diaphragmatic weakness is considered to be the major reason contributing to the reduction of lung function of people after stroke [11]. Diaphragm, as the primary inspiratory muscle, accounts for approximately 75% of the airflow into the lungs during quiet breathing [12]. However, most people after stroke have diaphragmatic weakness due to the loss of central motor control, leading to paralysis of the diaphragm in the contralateral side [13, 14]. Diaphragmatic weakness can lead to asymmetric respiratory chest movements, changes in the respiratory mechanism, and consequently reduced lung function [11, 14, 15]. The thickness of the affected diaphragm of patients after sub-acute and chronic stroke was found only to have nearly 50% of those of healthy individuals [13]. Contraction of diaphragm fibers can be quantified by the diaphragmic thickening fraction (DTF), determined by "(thickness of the diaphragm at end inspiratory - thickness at end expiratory)/thickness at end expiratory" [16]. A reduction in diaphragmatic contraction was reported to be significantly correlated with decreased lung function (e.g. FVC, FEV1, MIP, PEF) in people after stroke [13, 17].

Inspiratory muscle training (IMT) is an intervention designed for improvement of inspiratory muscle (including the diaphragm) strength and endurance in healthy adults and patients suffering from different diseases (e.g. Parkinson, chronic obstructive pulmonary disease, cardiovascular disease) [18-21]. Regarding the IMT training protocol, the commonly reported training intensities varied between 30-80% of MIP in people after stroke [22-24]. Although the diaphragm is the primary inspiratory muscle, the use of IMT may not help improve the diaphragmatic contraction under some circumstances. Previous studies suggested that, in healthy adults and people receiving mechanical ventilation, when inspiratory load exceeds the capacity of the diaphragm (higher than 60% MIP), the accessory respiratory muscle (in particular the sternocleidomastoid muscle) would be activated dramatically, which would increase work of breathing and fatigue [25, 26]. Other studies also reported that high IMT intensity may have the risk to increase the work of breathing thereby leading to fatigue [27] or resulting in a decrease, rather than increase, in surface electromyography signals in diaphragmatic activity [27]. It is postulated that IMT recruits accessory muscles such as sternocleidomastoid rather than diaphragm during the practice [24]. Hence, evaluation of effectiveness of IMT on DTF in people after stroke and further investigation on determining an optimal training protocol including inspiratory effort and training intensity for recruiting diaphragmatic activities in the stroke population are warranted.

Aims of the study:

To determine the optimal training intensity that is associated with the strongest diaphragmic thickening fraction in people after stroke.

Project significance:

Outcomes of this project study will fill the existing knowledge gaps in the understanding of the relationship between inspiratory pressure and recruitment of diaphragmatic muscles in people after sub-acute and chronic stroke.

4. Research methodology

Study design:

This study will be a cross-sectional study.

Participants:

Individuals who have had a stroke and healthy adults will be invited to participate in this study.

Participants recruitment for people after stroke:

The inclusion criteria are: (1) age $\geqslant$ 18 years; (2) clinically diagnosed with ischemic and/or hemorrhagic stroke; (3) duration of stroke range from 1 month to 12 months after diagnosis; (4) had no facial palsy, which could prevent proper labial occlusion; (5) had not undergone thoracic or abdominal surgery; (6) could understand and follow the verbal instruction; (7) stable cardiac conditions; (8) no previous history of respiratory problems.

The exclusion criteria are: (1) acute myocardial infarction or acute heart failure; (2) acute pain on chest or abdominal; (3) with the clinical signs of significant pulmonary disease; (4) consciousness impaired; (5) patient with nasal feeding tube, tracheal tube and/or any condition, which prevent the measurement or training.

Recruitment of healthy participants:
The inclusion criteria are: (1) age ⩾ 18 years; (2) had not undergone thoracic or abdominal surgery; (3) no previous history of respiratory problems.

The exclusion criteria are: (1) acute myocardial infarction or acute heart failure; (2) acute pain in the chest or abdominal; (3) with the clinical signs of significant pulmonary disease; (4) unstable hypertension, arrhythmias, or unstable cardiovascular conditions, such as fluctuations in blood pressure and heart rate, indicate poor cardiac prognosis or the need for vasopressor medications; (5) pregnant.

Ethical approval:
Ethical approval will be sought from the Research Ethics Committee of Hong Kong Metropolitan University and Shenzhen Second People's Hospital.

Sample size calculation:
Sample size calculation will be based on the use of repeated-measures analysis of variance (ANOVA) using the G*Power 3.1. Considering an effect size of 0.2, an alpha error probability of 0.05 and a power of 0.80, the estimated sample size is 54 participants. An additional 20% of the sample size will be included to account for attrition. The final estimated sample size will be at least 130 participants (65 for each cohort).

Procedure:
The nature of the study will be explained to the participants and written informed consent will be obtained. Demographic data of each participant including age, sex, height, weight, BMI, percent body fat, lean body mass, and smoking history will be recorded. For people after stroke, the stroke type (cerebral infarction, intracerebral hemorrhage), medical history (hypertension, diabetes mellitus, cardiovascular disease, lipidemia, kidney disease) will also be recorded. The National Institutes of Health Stroke Scale (NIHSS), Modified Rivermead Mobility Index (MRMI), Modified Rankin Scale (mRS), Barthel index (BI) will be retrieved from the patient's medical record.

Participants will be invited to attend the cardiopulmonary laboratory of the hospital at a time > 2 hours after a light meal. Participants are requested to avoid caffeine-containing products, nicotine, alcohol and vigorous exercise for at least 12 hours before attending the laboratory.

Each participant will be asked to perform the lung function test to measure the FVC, FEV_1, and MIP after including the study. After completing the baseline lung function test, participant will be required to sit on the chair and rest for 15 minutes, after that, two trained researchers will use two identical ultrasonography to measure the left and right diaphragmatic thickness simultaneously at quiet breathing, the end of tidal inspiratory and the end of tidal expiratory, respectively. 3 ultrasonic images will be captured to calculate the mean diaphragmatic thickness of each breathing status. In order to determine the inter-rater reliability of the measurement of ultrasonography of

those two trained researchers, 20 participants will be chosen out randomly to receive twice diaphragmatic thickness measurement at quiet breathing, the end of tidal inspiratory and the end of tidal expiratory. However, during the second measurement, those two trained researchers will change their position to measure the contralateral side diaphragmatic thickness independently.

After the baseline measurement, all participants will be requested to use a nose clip to hold the nose and breathe with the mouth through a pressure threshold inspiratory loading device (POWERbreathe, KH2, England). The inspiratory pressure will be set at 30%, 40%, 50%, 60%, 70%, or 80% of their MIP, in random order. Each MIP intensity protocol consists of 10 breaths. The procedure of IMT is followed by the published research protocols in people after stroke [28]. All MIP intensity protocols will be conducted in one day. A period of at least 10 minutes of rest time will be allowed between different protocols of contraction intensity (% MIP) on the same day [29].

Thickness of the left and right diaphragm (stroke and non-stroke), will be measured simultaneously by two identical ultrasonography at the end of maximum inspiration of 10 breaths of each MIP intensity protocol. The diaphragm thickness of both the left and right diaphragm at the end inspiratory breaths of the 10 breaths of each MIP intensity protocol will be recorded. Surface electromyography (sEMG) will be used to measure the activity of accessory muscle (sternocleidomastoid muscles) during each MIP intensity protocol. The % of MIP that associates with the highest calculated DTF and a relatively stable change in the activity of accessory muscles will be considered an appropriate training intensity for further IMT protocols.
The self-perceived exertion level will also be determined by the Rate of Perceived Exertion Borg scale at the end of each MIP intensity protocol [30].

All research procedures will be repeated again after 2 days to examine the test-retest reliability of ultrasonographic measurements of DTF.

Outcome measures:
Lung function
FVC, FEV1 will be measured by using a spirometer (XEEK, X1, China), MIP will be measured by another device (Power Breathe, KH2, England). During the lung function tests, participants need to remain in the upright sitting position with a nose clip that tightly fits the nostrils. To record MIP, participants will be instructed to perform inspirations against an obstructed airway within the mouthpiece at near-residual volume. All lung function tests require each participant to inhale and exhale as hard and as fast as possible, then complete the cycle by inspiring as hard and rapidly as possible. Each test will be required to be performed at least three times, with each test not differing by more than 5% or 100 milliliters. The largest value obtained from the three executions will be used in the final analysis [31]. All tests will follow the recommendations proposed by the guidelines of the lung function tests [32].

Power Breathe (KH2)
The Power Breathe KH2 is an inspiratory muscle training device designed to improve the strength and endurance of inspiratory muscles (as shown in picture 1) [33]. It consists of a mouthpiece and a breathing valve. The valve is calibrated to provide a specific resistance during inhalation. The resistance can be adjusted on the device according to the IMT prescription. Participant needs to inhale through the mouthpiece

and drive their inspiratory muscle to contract against the external inspiratory breathing load.

(a) (b) (c)

(a) the lateral side of Power Breathe (KH2).
(b) the front side of Power Breathe (KH2).
(c) the back side of Power Breathe (KH2), the top part (blue color) is the breathing valve.

Muscle activity of sternocleidomastoid muscles

Surface electromyography (sEMG) (Telemyo 900 from Noraxon USA) will be used to measure accessory inspiratory muscle (sternocleidomastoid muscles) activity during each MIP intensity protocol. The electrodes for sEMG will be attached to the bilateral sternocleidomastoid muscles (SCM). The locations of the electrodes will be placed at the mid-point of the left and right SCM muscles to measure the muscle activity of IM (shown in figure 1(a)). The sEMG responses will be amplified (gain × 1,000); the band-pass will be filtered between 5 and 500 Hz, and digitized at a sampling rate of 1 kHz by using an analogue to-digital converter. The acquired data will be later analyzed using matching software.

The measurement of diaphragmatic thickness

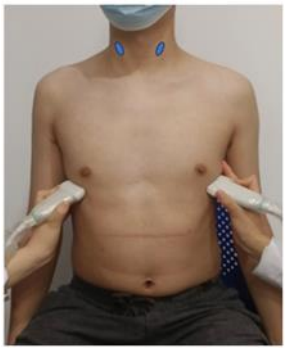
Diaphragmatic thickness will be measured by the ultrasound machine (Mindray 9, Shenzhen, PRC). Ultrasonography is a portable, noninvasive emerging technique allowing easy and rapid estimation of muscle thickness [34, 35]. It not only can avoid the risk of exposure to radiation in medical facilities but also the reliability in providing a clear visualization of the muscle morphology at the zone of apposition [35, 36].

For consistency, all measurements will be conducted using the same transducer probe (model SuperLinear SL18-8; SuperSonic Imagine) with a bandwidth of 5 -18Hz of B mode. Ultrasonography will be performed by two trained physiotherapist who operates the machine under the guidance of a radiologist who is trained to use diagnostic ultrasound.

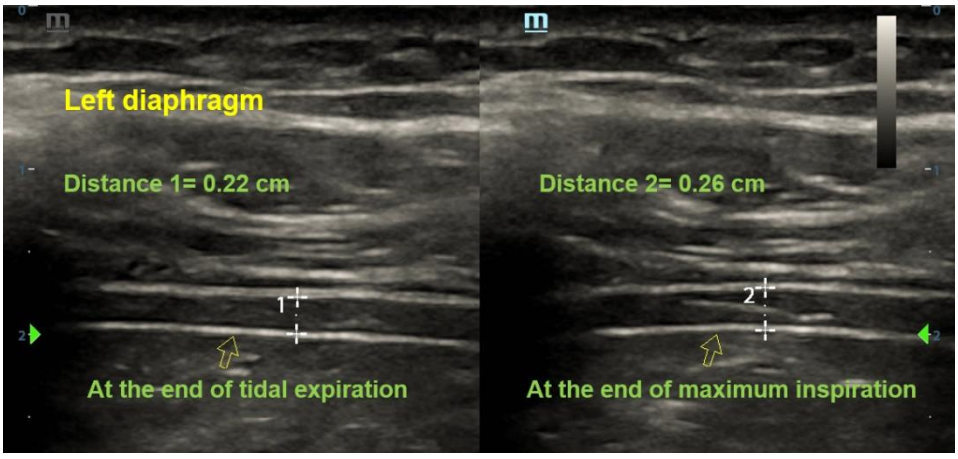
Participant needs to remain in the upright sitting position on the chair during the measurement procedure. To measure the right diaphragmatic thickness, the transducer probe will be placed in the right intercostal space above the eighth or ninth intercostal space, between the antero-axillary and mid-axillary lines with a 45° angle tilt between

the surface of the abdominal wall in the cephalic direction, where the diaphragm is most suitable visualized (shown in figure1(a)). To measure the left diaphragmatic thickness, the transducer will be placed in the left intercostal space above the eighth or ninth intercostal space, between the anterior subcostal region between the anterior and midaxillary lines (shown in figure 2(a)). The diaphragm is observed as a three-layered structure, with the hypoechogenic muscular layer bordered by echogenic layers—the peritoneum and the diaphragmatic pleurae (as shown in figure2(b) and figure2(c)).

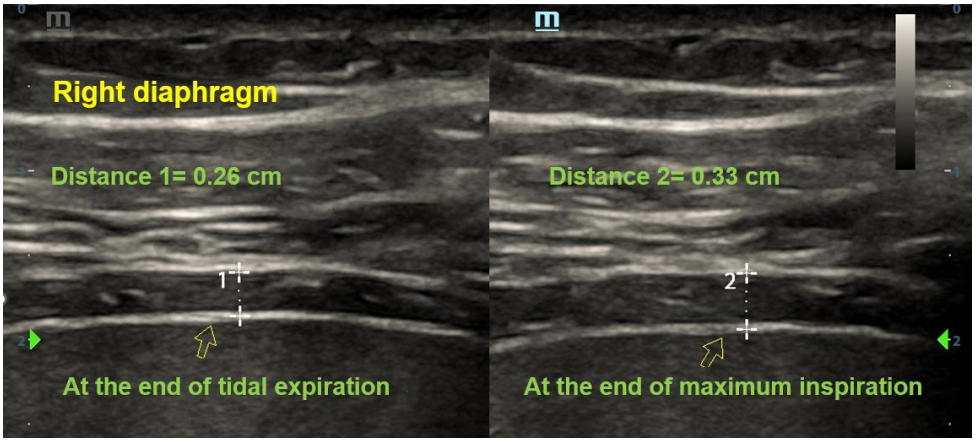
Figure 1 The placement of transducer probes, surface electromyography electrodes and image of the diaphragm



(a) The placement of transducer probes when measure of the right and left diaphragm by ultrasonography in people after stroke (left hemiplegia); The blue points represent the locations of surface electromyography electrodes on the sternocleidomastoid muscles.

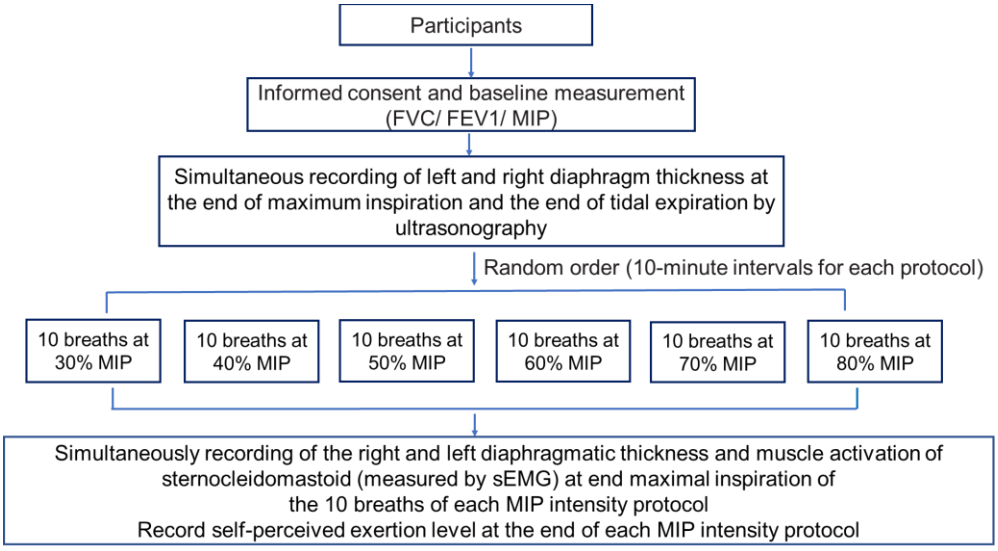


(b) Diaphragmatic thickness images of the left diaphragm (stroke side) at the end of tidal expiration and the end of maximum inspiration at 30 %MIP.



(c) Diaphragmatic thickness images of the right diaphragm (non-stroke side) at the end of tidal expiration and the end of maximum inspiration at 30 %MIP.

Research flow chart



Reporting of adverse event:

Though various measures will be taken to minimize the risk of adverse event throughout the study, any adverse events that might happen will be recorded.

Statistical analysis:

All data will be reported as mean ± standard deviation (SD) unless otherwise indicated. Demographic data and clinical characteristics for all participants will be summarized using descriptive statistics. Repeated-measures ANOVA will be used to compare the differences of DTF and sEMG parameters determined at each inspiratory pressure separately. Intraclass correlation coefficient will be used to determine the inter-rater reliability of two ultrasonic operators and examine the test-retest reliability coefficient of ultrasonographic measurements of DTF recorded with different inspiratory pressures.

P value will be set at 0.05. All data will be analyzed using the IBM SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp).

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