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ORIGINAL RESEARCH **CARDIOVASCULAR ANESTHESIA**

Effects of pre- and post-operative active cycle of breathing technique as an add-on to routine chest physical therapy on respiratory parameters in coronary artery bypass graft patients

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ABSTRACT

Background & Objective: When someone has coronary artery disease, surgeons may perform a coronary artery bypass graft (CABG) to create a new route for blood to reach the narrowed heart arteries. CABG procedures may increase the risk of patients having pulmonary issues after surgery. The usefulness of active cycle of breathing technique (ACBT) in patients who undergo CABG to lower the risk of lung problems has not been widely recognized or experienced in Pakistan. Therefore, the purpose was to determine the effects of pre and postoperative ACBT on oxygen saturation (SpO_2), arterial blood gas (ABG), and respiratory rate (RR) after coronary artery bypass graft surgery (CABG).

Methodology: The randomized controlled trial took place from March to May 2024 at the Faisalabad Institute of Cardiology (FIC) in Faisalabad. The elected 72 patients both men and women of ages 35 to 65 were randomized using lottery method to place equal numbers of participants in each group (n = 36 each). ACBT was applied in addition to routine chest physical therapy in the intervention group, while the control group received only routine chest therapy. Both patient groups had their arterial blood gases (ABG), oxygen saturation (SpO_2) and respiratory rate (RR) checked at baseline and upto five days after surgery (daily).

Results: There were significant differences between the groups for RR, SpO_2 and $PaCO_2$ over all the postoperative days ($p < 0.001$). Nevertheless, there was no noticeable difference in PaO_2 on Day 3 (P = 0.977), in HCO_3 on Day 3 (P = 0.145) and 4 (P = 0.84), nor in pH on Days 2 (P = 0.158) and 3 (P = 0.017). SpO_2 in the control group was 95 (95–96) at the start, climbed to 99 (98–99.4) on Day 1, then dropped to 95 (95–96) by Day 5. RR went up to 28 (27.25–30) on Day 5. For the intervention group, the SpO_2 went from 95 (95–96.75) at the beginning to 99 (98.25–100) by Day 5 and the RR was reduced from 30 (19–31) on Day 1 down to 22 (20–24) on Day 5. Within the control group, SpO_2 went down to 81% and RR went up to 36 ± 3.75 on Day 3 which indicates the respiratory system had not returned to normal as fast as the intervention group.

Conclusion: The findings of our study suggest that 'active cycle of breathing technique' is more effective than the routine chest physical therapy alone for improving hemodynamic and respiratory parameters including ABG, RR, and SpO_2 after CABG.

Abbreviations: ABG: arterial blood gas, ACBT: Active cycle breathing technique, CABG: Coronary artery bypass graft, RR: respiratory rate

Keywords: Active cycle breathing technique; Arterial blood gasses; Coronary artery bypass graft; Oxygen saturation; postoperative; pulmonary complications; Respiratory rate

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1. INTRODUCTION

According to the World Health Organization (WHO), cardiovascular diseases account for approximately 17.9 million deaths each year, constituting roughly 32% of the global mortality rate.[1] Coronary artery disease (CAD) has become an epidemic in recent years in Southeast Asia, particularly in Pakistan.[2] Heart bypass surgery also known as coronary artery bypass grafting (CABG) is a surgical procedure that enhances the blood flow to ischemic cardiac tissues by creating bypasses around the clogged arteries.[3] Individuals who have had bypass surgery are more likely to develop pulmonary complications and oxygenation disorders.[4] CABG is an invasive surgery, with several risks associated with intubation, including airway damage, infection, pain, shortness of breath and the impact of anesthesia leads to various complications.[5] The pulmonary complications include arterial blood gas disturbance, respiratory failure, decreased lung volumes and capacities, and respiratory muscle dysfunction.[6]

The incidence of pulmonary complications after surgery is 30 to 60%.[7] Furthermore, certain preoperative factors play a role in postoperative pulmonary complications including pre-existing lung conditions, older age, smoking history, obesity, obstructive sleep apnea (OSA), heart failure, and inadequate nutrition.[8] Increased levels of blood C-reactive protein (CRP), cortisol, and inflammatory markers significantly affect respiratory muscles, leading to reduced protein synthesis.[9] When respiratory muscle contractions are compromised, ventilation is impaired, resulting in decreased respiratory volumes.[10] Initiating a rehabilitation program after CABG surgery is an essential component of the management plan.[11] Cardiopulmonary rehabilitation provides education and counseling to individuals with heart conditions minimize their risk of future heart issues by enhancing physical fitness and overall health.[12] Primary methods in cardiopulmonary rehabilitation are active cycle of breathing techniques, deep breathing exercises, percussion, and the use of an incentive spirometer.[13] These techniques may increase lung volumes and capacity, oxygen saturation.[14]

ACBT is a cycle of breathing exercises commonly used to help patients with respiratory diseases and postoperative conditions improve their lung functions.[15] Literature reports, ACBT and chest physiotherapy has the potential to reduce post-operative pulmonary complications.[16] ACBT combined with routine chest physiotherapy, increases ventilation, raises oxygen saturation, reduces discomfort or pain, and prevents respiratory acidosis.[17] The combined use of ACBT and incentive spirometer are frequently used in CABG patients.[18] Derakhtanjani *et al.* conducted a study which concluded that ACBT and routine chest physical therapy both had equal effects on ABG, arterial oxygenation and pain after CABG.[19] Charity *et al.* and Westerdahl *et al.* also concluded in their studies that evidence supporting the positive outcomes in the use of ACBT as breathing exercise in improving pulmonary functions in CABG patients.[20,21] According to another study who compared ACBT with routine chest physical therapy proved that ACBT is more beneficial in improving respiratory rate, oxygen saturation and chest expansion.[22]

This study aims to determine the effects of the ACBT and routine chest physical therapy in reducing postoperative pulmonary dysfunction while closely monitored arterial blood gas, oxygen saturation, and respiratory rate in coronary artery bypass graft patients. The effectiveness of the ACBT contributes to the development of evidence-based practice for the preoperative and postoperative treatment of CABG patients. There is still lack of evidence and only few studies available on ACBT, showed differences and effects of the technique over these parameters.

2. METHODOLOGY

Between December 2023 and May 2024, at Faisalabad Institute of Cardiology, this randomized controlled trial was carried out. The trial was recorded on ClinicalTrials.gov (with identifier NCT06705556) once approval was given by the University of Faisalabad's Research and Ethics Committee (Ref: TUF/IRB/292/24).

Figure 1: Patient performing ACBTs

The research considered both men and women of 35 to 65 years who had a medical history of stable coronary artery disease for at least 15 years and were taking part in elective coronary artery bypass (CABG) surgery. Patients having unstable vitals, preoperative lung problems, COPD, emphysema, asthma, severe atelectasis, renal problems or need ventilator support for more than 48 hours after the procedure, were excluded.

Using OpenEpi software and setting the significance level to 95% (Z_1-α/2) and the power to 80% (Z_1-β), mean change expected in Group A is 23.6 (SD = 2.32) and in Group B is 25.71 (SD = 3.4).[23] This gave a required sample size of 30 participants per group. After adding 20% dropout rate, the sample size rose to 36 participants in each group and there were 72 participants overall. The technique selected was non-probability purposive sampling, followed by a screening of people using set criteria. Once written informed consent was given, the eligible participants were randomly assigned (1:1) to the interventional and conventional groups in a randomizing program (lottery method). Patient enrolment was ensured by an independent researcher not involved in patient care and allocation concealment was achieved utilizing sequentially numbered, sealed, opaque envelopes. Selection bias was avoided because the envelopes were opened after a consent inclusion decision was made.

The study was single-blinded in which the assessor was blind. A self-administered questionnaire was designed to collect demographic data including age, gender, BMI, hypertension, diabetes and smoking status. An oxygen saturation reader (pulse oximeter) was attached to the patient to show how much oxygen she was getting and a cardiac monitor was used to read her breathing rate whereas Blood gas analyzer machine was used to analyze ABGs. Before taking the sample, the procedure was described to the patient, and consent was taken.

Palpate the patient's brachial artery over the cubital fossa, clean the surface with an alcohol wipe, and administer local anesthetic subcutaneously over the target site. Remove the cover from the ABG needle and flush the heparin from a syringe. Support the patient's arm over the pillow and insert the ABG needle at an angle of 30-45°. When the ABG needle was self-filled and a desired amount of blood was collected, the needle was removed and immediate pressure was applied over the puncture site for about 3-5 minutes. Hold the gauze with tape, and keep applying pressure. ABG needle was removed from the syringe and discarded into a bin. A cap was placed over the ABG syringe. The ABG syringe was labeled with the patient's name. The ABG sample was obtained under local anesthesia once at baseline, after surgery an arterial line was placed in the patients' brachial artery through which the sample was collected and analyzed using the ABG analyzer machine. Pulse oximeter was used to measured oxygen saturation and cardiac monitor was used to measure respiratory rate.

Table 1: Descriptive statistics for demographic data

Variables		Interventional group	Control group	P-value
Age		53.64 ± 5.82	55.75 ± 4.19	> 0.05
Gender	Male	25 (69.4)	29 (80.6)	> 0.05
	Female	11 (30.6)	7 (19.4)	
Smoking		13 (36.1)	25 (69.4)	> 0.05
Hypertension		24 (66.7)	26 (72.2)	> 0.05
Diabetes Mellitus		28 (77.8)	26 (72.2)	> 0.05

Data are presented as mean ± SD or n (%0; P < 0,05 is significant

2.1. Interventions

2.1.1. Control Group

The routine chest physical therapy was performed 3 days before surgery at the preoperative bay and 5 days after surgery in the ICU with 3 sessions each day. Preoperative chest physical therapy sessions were followed as: Incentive spirometry (3 sessions per day with 10 repetitions each time), Percussions (3 sessions per day with 20 repetitions) and 3-minutes walking (2 sessions per day).

Table 2: Comparison of pre and postoperative variable values within the Control Group- Friedman Test

Measure-ments	Median Interquartile range for all variables					
	pH	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	HCO_3 (mmol/L)	RR (/min)	SpO_2 (%)
Preop	7.42 ± 0.05	42 ± 2	85 ± 0	25 ± 4	18.5 ± 1.8	95 ± 1
Day 1	7.34 ± 0.10	33 ± 2	110 ± 14	21 ± 1	16 ± 2	99 ± 1.4
Day 2	7.40 ± 0.12	34 ± 2.8	80 ± 0	22 ± 1	20 ± 1.5	81 ± 2
Day 3	7.45 ± 0.00	47 ± 3	90 ± 0	25.5 ± 4	36 ± 3.8	90 ± 10
Day 4	7.50 ± 0.06	44.5 ± 2.5	90 ± 10	24 ± 3.8	30 ± 1	92 ± 2
Day 5	7.45 ± 0.05	35.2 ± 0.8	110 ± 10.8	27.5 ± 3	28 ± 2.8	95 ± 1

All comparisons across days were significant at the level of statistical probability < 0.001

Table 3: Comparison of pre and postoperative variable values within the Interventional Group- Friedman Test

Variables		Preoperative	Day 1	Day 2	Day 3	Day 4	Day 5
pH	Median (IQR)	7.45 (7.4-7.45)	7.38 (7.30-7.32)	7.4 (7.40-7.45)	7.45 (7.40-7.45)	7.45 (7.40-7.45)	7.4 (7.40-7.45)
	Rank	3.94	1.44	3.58	4.11	4.33	3.58
$PaCO_2$	Median (IQR)	42.50 (41-43)	35 (34-35)	45 (45-45)	48 (48-49)	38 (37.25-41.75)	34.14 (33-35)
	Rank	3.82	2.07	4.99	5.93	2.71	1.49
PaO_2	Median (IQR)	85 (85-85)	120 (115-120)	95 (90-110)	90 (85-95)	110 (105-120)	120 (120-120)
	Rank	1.28	5.06	3.03	2.03	4.25	5.36
HCO_3	Median (IQR)	26 (22.25-28)	26 (22.25-28)	27 (26-28)	26.50 (25.25-28)	24 (22-25)	22 (22-23.50)
	Rank	3.81	3.99	4.21	4.21	2.81	1.99
RR	Median (IQR)	18 (18-19)	30 (19-31)	30.50 (30-31)	33 (31-35)	28.50 (27.25-29.75)	22 (2-24)
	Rank	1.21	3.51	4.60	5.63	3.69	2.36
SpO_2	Median (IQR)	95 (95-96.75)	98 (96-98)	95 (95-95.75)	93 (92-95)	98 (97-98)	99 (98.25-100)
	Rank	2.82	3.94	2.75	1.81	4.21	5.47

All comparisons across days were significant at the level of statistical probability < 0.001.

The preoperative chest physiotherapy treatment plan was continued after CABG surgery. In addition to these, the following were included postoperatively: Nebulization-three times a day, 3-5 minutes walking- twice a day (as per patient condition). The "Life Care" incentive spirometer device was used in the current step-up. The one-ball and 3 balls (tri-flow) were over 600cc to 1200cc volume per second IS used. The IS were performed during the sessions and afterward, patients and their attendants were guided to repeat it every 2 hours to maintain changes in RR, ABG, and SpO_2 over time. The $PaCO_2$ has a direct relation with RR and an inverse relation with pH in the case of increased $PaCO_2$ above 45mmHg then 1 session of blow bottle exercise was performed which had a direct effect on pH and respiratory rates have returned to normal.

2.1.2. Interventional Group

Patients were instructed to perform ACBT in addition to routine chest physical therapy, before and after surgery.[24] In ACBT, they were instructed to do "6-8 relaxed breaths" (normal tummy breaths), then taking 3 deep breaths and holding them for 3 seconds at the bottom of their lungs. Followed by this, they repeated 6–8 normal relaxed breaths, again took 3 deep breaths at bottom of lungs with hold for 3 seconds and ended with another set of 6–8 relaxed normal tummy breaths. The last step of the cycle was "huffing-followed by coughing" where huffing was to make two medium breaths out slowly through the mouth as if fogging-up a mirror. Number of sessions, repetitions and nebulization medicines given to the intervention group were the same

Table 4: Comparison of pre and postoperative Arterial Blood Gas between Groups

Variables		Control Group	Intervention Group	P value
Preoperative pH	**612**	**7.42 (7.4-7.45)**	**7.45 (7.4-7.45)**	**0.639**
Day 1	439	7.34 (7.3-7.4)	7.38 (7.30-7.32)	0.012
Day 2	532	7.4 (7.33-7.45)	7.4 (7.40-7.45)	0.158
Day 3	486	7.45 (7.45-7.45)	7.45 (7.40-7.45)	0.017
Day 4	215	7.5 (7.45-7.51)	7.45 (7.40-7.45)	< 0.001
Day 5	415	7.45 (7.40-7.45)	7.4 (7.40-7.45)	< 0.001
Preoperative $PaCO_2$	**591**	**42 (41-43)**	**42.50 (41-43)**	**0.516**
Day 1	248	33 (32-34)	35 (34-35)	< 0.001
Day 2	.00	34 (32.25-35)	45 (45-45)	< 0.001
Day 3	391	47 (45-48)	48 (48-49)	< 0.001
Day 4	22	44.5 (44-46.50)	38 (37.25-41.75)	< 0.001
Day 5	404.5	35.22 (35-35.75)	34.14 (33-35)	< 0.001
Preoperative PaO_2	**615**	**85 (85-85)**	**85 (85-85)**	**0.502**
Day 1	298.5	110 (105-119)	120 (115-120)	< 0.001
Day 2	27	80 (80-80)	95 (90-110)	< 0.001
Day 3	645	90 (90-90)	90 (85-95)	0.977
Day 4	114	90 (85-95)	110 (105-120)	< 0.001
Day 5	191	110 (105-115.75)	120 (120-120)	< 0.001
Preoperative HCO_3	**578**	**25 (23-27)**	**26 (22.25-28)**	**0.431**
Day 1	124	21 (21-22)	26 (22.25-28)	< 0.001
Day 2	24	22 (21-22)	27 (26-28)	< 0.001
Day 3	522	25.50 (24-28)	26.50 (25.25-28)	0.145
Day 4	630	24 (22-25.75)	24 (22-25)	0.840
Day 5	177	27.50 (25-28)	22 (22-23.50)	< 0.001

Data presented as Median (IQR); Mann Whitney U Test was applied; P < 0.05 is significant

as those given to the control group. At the pre-surgery stage, ACBT was given three times per day and it continued to be done for up to five days in the ICU after CABG.

The ABG, SpO_2, and RR were assessed once at baseline prior to surgery. When the patients in both groups were stable after surgery and the endotracheal tube removed, the ABG, RR, and SpO_2 were monitored every 2 hours on postoperative days 1, 2, and 3, and every 4 hours on days 4 and 5 following the chest physical therapy interventions.

2.2. Statistical Analysis

Data was entered into a statistical package for social sciences (SPSS) version 21.00. Mean and standard deviation were calculated for numerical variables whereas frequencies & percentages were taken for qualitative variables. The normality of data was checked through the Kolmogorov Smirnov test and the results of this test showed that data were non-normally distributed therefore, nonparametric tests were used for further analysis. Within-group comparisons were assessed using Friedman's test. For between-group comparisons, the Mann-Whitney U test was utilized. A p-value equal to or less than 0.05 was considered significant

3. RESULTS

The Kolmogorov-Smirnov test indicated that respiratory rate, SpO_2 saturation, $PaCO_2$ levels, PaO_2 levels, HCO3,

Table 5: Comparative pre and postoperative oxygen saturation and respiratory rate between Groups

Parameters	Mann Whitney U Test	Control Group Median (IQR)	Intervention Group Median (IQR)	P value
Preoperative RR	**595**	**18.50 (18-19.75)**	**18 (18-19)**	**0.515**
Day 1	64	16 (16-18)	30 (19-31)	< 0.001
Day 2	18	20 (19.25-20.75)	30.50 (30-31)	< 0.001
Day 3	315	36 (34-37.75)	33 (31-35)	< 0.001
Day 4	277	30 (30-31)	28.50 (27.25-29.75)	< 0.001
Day 5	39	28 (27.25-30)	22 (22-24)	< 0.001
Preoperative SpO_2	**622**	**95 (95-96)**	**95 (95-96.75)**	**0.481**
Day 1	232	99 (98-99.4)	98 (96-98)	< 0.001
Day 2	.00	81 (80-82)	95 (95-95.75)	< 0.001
Day 3	168	90 (81-91)	93 (92-95)	< 0.001
Day 4	.00	92 (92-94)	98 (97-98)	< 0.001
Day 5	33	95 (95-96)	99 (98.25-100)	< 0.001

Data presented as Median (IQR); Mann Whitney U Test was applied; P < 0.05 is significant

and pH were non-normally distributed (P < 0.05). Therefore, non-parametric tests; Friedman test and Mann-Whitney U test were applied. For pH levels, no significant difference was noted preoperative (P=0.639), but significant differences were observed on most postoperative days except for Day 2 (P=0.158) and Day 3 (P = 0.017).

Preoperative $PaCO_2$ did not differ significantly (P=0.516), but significant differences were found on all postoperative days (P < 0.001). Similarly, PaO_2 showed no difference preoperative (P=0.502) but found significant differences on all postoperative days except Day 3 (P=0.977). The HCO_3 mEq/L levels showed significant differences on most postoperative days, except for Day 3 (P=0.145) and Day 4 (P=0.840). Overall, the intervention group demonstrated better outcomes in respiratory rate, SpO_2, $PaCO_2$, PaO_2, HCO_3, and pH levels compared to the control group. In the control group, recovery of RR was significant, peaking at 36 (34–37.75) on the third day, while SpO_2 dropped to a minimum of 81 (80–82) on the second day before getting better by the fifth day.

The Friedman test revealed significant differences in all parameters over five days of the early postoperative period. For the intervention group, initially, the preoperative respiratory rate stood at 18 breaths per minute and showed a significant increase to 30 breaths per minute on Days 1, 2, and 33 on Day 3. Subsequently, it gradually returned to normal to 28 and 22 breaths per minute on Days 4 and 5, respectively. Friedman's test indicated that there were significant changes in oxygen saturation among the intervention group x2 (5) =97.409, (p <0.001).

Initially, the preoperative respiratory rate stood at 18 breaths per minute and showed a significant increase to 30 breaths per minute on Days 1, 2, and 33 on Day 3. Subsequently, it gradually returned to normal to 28 and 22 breaths per minute on Days 4 and 5, respectively with statistically significant differences.

Using Mann Whitney U test, in Table 4, comparison was done between both groups in terms of ABGs, RR, SpO_2. For pH levels, significant differences were observed at all postoperative days between the groups (P < .001), except for Day 2 (P = .158) and Day 3 (P = .017). In terms of $PaCO_2$ mmHg levels, significant differences were observed at all postoperative days (Day 1 to 5) between the groups (P < .001), except for the preoperative $PaCO_2$ mmHg (P = .516). For PaO_2 levels, significant differences were noted at all postoperative days between the groups (P < .001), except for Day 3 (P = .977).

Regarding HCO_3 mEq/L levels, significant differences were found on postoperative days 1, 2, and 5 between the groups (P < .001) while no significant differences were observed on Day 3 (P = .145) and Day 4 (P = .840). In comparison to the control group, the intervention group showed better outcomes in respiratory rate, SpO_2, $PaCO_2$, PaO_2, HCO_3, and pH levels after surgery.t has also been seen in Table 5 that there were no significant differences observed at preoperative SpO_2 between groups while after surgery the intervention group showed a better outcome on all postoperative days as compared to the control group (<0.001). There were no significant differences in the preoperative respiratory rate between groups ($P = 0.515$). However, significant differences were observed in respiratory rates at all postoperative days ($P < 0.001$).

4. DISCUSSION

The study demonstrates that active cycle of breathing technique as add on to routine chest physical therapy significantly improved respiratory parameters after CABG surgery except day 2 and 3. The improvements in respiratory parameters is demonstrated by the increase in arterial blood gas, respiratory rate and oxygen saturation. This study disclosed that both groups manifested improvement in restoring the normal oxygen saturation and ABGs, but intervention group showed little more improvement as compare to control group.

In the current study, ACBT as add on to routine chest physical therapy improved arterial blood gas, respiratory rate and oxygen saturation in post CABG patients. There were significant improvements of respiratory parameters from baseline to postoperative day 5th. In 2022, Nouman *et al.* studied the effects of ACBT with conventional treatment on FEV_1, SpO_2, RR and chest expansion. They found that ACBT is better in effects as compared to conventional physical therapy.[22] Another study found that ACBT and routine chest physiotherapy produced comparable improvements in arterial oxygenation, heart rate, and pain after CABG surgery, in contrast to our results demonstrate that ACBT yielded a significant improvement in ABG values and SpO_2 five days post-operatively.[19] There were statistical and clinical improvements in ABG, SpO_2 of both the groups signifying that the deep breathing in ACBT and incentive spirometer equally effective in preventing and treating complications after CABG surgery.[25] Similar results were found when compared ACBT with incentive spirometer after CABG surgery, that both interventions were the reason for improvements in respiratory functions.[26]

Yazdannik A *et al*. stated that PaO_2, SpO_2, and $PaCO_2$, significantly improved with routine chest physical therapy after CABG surgery.[27] However, current study assesses the effects of ACBT and routine chest physiotherapy techniques on PaO_2 $PaCO_2$ and SpO_2, HCO_3, and RR. Another study increases oxygen saturation with help of breathing exercises.[28] Mordianet al. concluded that combined use of deep breathing exercise and incentive spirometer improved ABG and SpO_2, after CABG surgery.[29]

A few studies available regarding ACBT and routine chest physical therapy before and after surgery for the treatment of ABG, RR and SpO_2 simultaneously, even there is not a single study available who suggest ACBT over routine chest physical therapy. And none of the above studies mentioned the use of these techniques as a treatment option before and after CABG surgery.

5. Limitations and Recommendations

Its finding might be limited by the fact that the research was done at one hospital with a relatively small group of patients. Older adults and those having other types of cardiothoracic surgery were also not included in this study since the focus was only on elective CABG for adults aged 35 to 65. Studies should be conducted including data from more healthcare centers, with a larger and different mixture of patients such as those over 65 and those having cardiothoracic surgery. It would help the research be more broadly applicable and aid in advancing physical therapy for those needing postoperative care for the heart.

6. CONCLUSION

This study concluded that ACBT is better in effects as compared to routine chest physical therapy for improving arterial blood gas, oxygen saturation and respiratory rate of all postoperative days except day 2 and day 3. Both ACBT and routine chest physical therapy improved respiratory parameters. But as far as improvements in respiratory parameters there is significant difference after surgery in patients receiving ACBT intervention.

7. Data Availability

Available upon request from the corresponding author.

8. Conflict of interest

The authors declare they do not have any conflict of interest.

9. Funding

Self-funded study

10. Authors' Contribution

SB: Conception, design & drafting of the work.
HSA: Reviewed & revised critically for important intellectual content.
ZA: Proofreading of final submitted manuscript.
NZ: Interpretation of data.
MAN: Analysis of data.
ZS: Drafting and Revision of work.

All authors had approved the final manuscript for submission and declare that the manuscript has not been previously published or submitted anywhere else.

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