EARLY REHABILITATION IN PATIENTS FOLLOWING COLORECTAL RESECTION: EFFECTIVENESS OF YOGIC BREATHING AND MANUAL THERAPY VS CONVENTIONAL CARE

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KEYWORDS: colorectal surgery, early rehabilitation, yogic breathing exercises, manual diaphragm release technique.

ABSTRACT

Introduction *Early rehabilitation programs have become an important focus of perioperative management after colorectal surgery with aims of improving patient care, reducing complication rates, and shortening hospital stay following colorectal surgery. The aim of this study is to evaluate the efficacy and safety of Yogic Breathing and Manual Diaphragm Release Technique in early rehabilitation of patients following surgery to remove colorectal cancer.*
Methods *A total of 40 patients with colorectal cancer who underwent colorectal resection were randomly assigned to receive either the experimental protocol (experimental group, EG: n 20 average age 66,5 years) or the stansard postoperative care (standard group, SG: n 20 average age 66 years). In all subjects, postoperative outcomes after seven sessions were: peripheral oxygen saturation of capillary blood hemoglobin (SpO2), heart rate (HR), Activities of Daily Living (ADL), Short-Form 12 (SF-12) questionnaire, Visual Analogue Scale (VAS) and pulmonary functionality (spirometer).*
Results *The length of postoperative hospital stay and ventilation hours were shorter in patients receiving the experimental protocol compared with those receiving the conventional postoperative care. A better pulmonary functionality, which is fundamental for reducing lung complications, was recorded in patients receiving the experimental program than in those receiving conventional care mainly in the percentage of Forced Expiratory Volume in 1st second. A significantly improvement of SPO2 and a greater decrement of HR was observed in the EG. To compare with SG, SF12 score after experimental protocol showed an improvement of quality of live. There was no significant difference in ADL score when the two groups were compared. Patients who received the experimental protocol compared with those receiving the standard postoperative care showed a significantly reduction of discomfort after surgery.*
Conclusion *Our experience demonstrated that there is the place for cooperation between Yogic Breathing and Manual Diaphragm Release Technique after colorectal surgery. The comparison between the groups showed that the SG has been in a favorable clinical condition to prevent PPCs than the CG group. Thus, this new approach could be used as a component of the management of patients who have undergone colorectal surgery for cancer.*

INTRODUCTION

Colorectal cancer is one of the leading causes of morbidity and mortality worldwide. Certainly, it is the third most common cancer diagnosed in men and the second most common cancer in women (Ferlay J, 2015). Despite advances in surgical care, colorectal surgery is associated with a high risk of morbidity and mortality in comparison to other kind of surgery specialties (Alves A, 2005). Indeed, post-operative complications could range from minor complications with minimal impact on length of stay to severe complications requiring intensive care stays and could affect patients' quality of life (Marinatou A, 2014). In an era of diminishing hospital resources and increasing medical costs, the reduction in postoperative stay has become a major focus to optimize utilization of healthcare resources (Gouvas N.,2009). To reduce that, fast-track rehabilitation programs integrate a range of perioperative interventions proven to maintain physiological function and facilitate postoperative recovery (Rosenthal MB, 2006). The positive effects of yoga have been investigated in a number of cancer patient and survivors (cancer survivors are individuals who have completed cancer treatment). Results from these evidences suggest that yoga is a feasible intervention for a wide range of cancer patients and survivors (Bower J. E, 2005). One of the Yoga practice is Pranayama. It is the art of controlling breath which integrates the mind and body. Thus, it produces many systemic effects in the respiratory functions and psycho-physical of the body (Mishra SP., 1997; Shankarappa V., 2012). In addition, Manual Diaphragm Release Technique improves diaphragmatic mobility, exercise capacity and inspiratory capacity, mainly in patients with chronic obstructive pulmonary disease. (T. Rocha, 2015). Although medical benefits of Yoga breathing and Manual Diaphragm Release Technique are recognized, there is an apparent lack of studies on the use of them in postoperative rehabilitation protocols. The aim of this research is to evaluate the effects of early rehabilitation, Yogic Breathing and Manual Diaphragm Release Technique in patients after surgical treatment of colorectal cancer, respecting their functional recovery and quality of life.

METHODS

Study Design

The study was conducted by Division of General Surgery at the Hospital "Santo Spirito" of Pescara. Any patients scheduled for elective laparoscopic and ro-

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botic (daVinci xi) colorectal resection were recruited from the Division of Abdominal Surgery of the same Hospital. They were diagnosed with primary colorectal cancer in Stages I -II- III and candidates for surgical tumor removal, that was judged as the best curative option. Forty individuals (20 males and 20 females, Caucasians) age from 28 to 80, overage 66 (smokers and non-smokers) were selected for the study. They were randomly assigned to two groups of 20 participants each: the standard group (SG) treated by usual early postoperative mobilization (table 1) and the experimental group (EG) treated by early postoperative mobilization added to Yogic Breathing and Manual Diaphragm Release (table 2).

Table 1 Demographics of SG selected for colonic resection

Count	20		
Age (years)	66 (± 14)		
Sex	Male	9	45%
	Female	11	55%
BMI (M_a)	25,2 (±4,8)		
ASA	I	1	5%
	II	8	40%
	III	11	55%

Table 2 Demographics of EG selected for colonic resection

Count	20		
Age (years)	66,5 (± 10,2)		
Sex	Male	11	55%
	Female	9	45%
BMI (M_a)	27,6 (±3,9)		
ASA	I	3	15%
	II	7	35%
	III	10	50%

Inclusion criteria included: age ≥18 and ≤ 80 years, no preoperative chemotherapy or radiotherapy, American Society of Anesthesiologists (ASA) grade I/II (Stefan De H,2018). Pre-operative evaluation of adults undergoing elective noncardiac surgery. Body Mass Index (BMI) 17.5–27.6 kg/m2. The exclusion criteria included: no eligibility for colorectal surgery or metastases, ASA grade III / IV, acute diseases and comorbidities (neurological, cardiovascular and pulmonary) incompatible with the study protocol. All patients underwent elective open colorectal resection with combined tracheal intubation and general anesthesia.

Study Procedures and Outcome Measures

The groups were evaluated at two time points: T0 (baseline) at admission to the Division of Abdominal Surgery and T1 (after surgery at the discharge). All patients were subjected to medical examination to delineate the characteristics and the eligibility to colorectal surgery. To evaluate pulmonary function was used a Spirolab III (MIR Medical International Research, Roma, Italy), a self-calibrating computerized spirometer that fulfills the criteria for standardized lung function tests. Before and after surgery, the subject was instructed to take maximum inspiration and blow into the mouthpiece as rapidly, forcefully, and completely as possible. A tight seal was maintained between lips and mouthpiece. Patients performed three tests in each evaluation, and the best of the three was taken into account for further analysis. All expressed as % of predicted values, the parameters chosen were: Forced vital capacity (FVC) – the amount of air that can be forcibly exhaled from the lung after taking the deepest breath possible. FVC ≥ 80% predicted, calculated from age, height, weight, gender, and ethnic group, was considered the norm. Forced expiratory volume in 1 s (FEV1) – the amount of air exhaled in 1 s, which was taken as a measure of airflow limitation. FEV1 ≥ 80% predicted was considered the norm (Ardestani ME, 2014). In addition, peripheral oxygen saturation (SpO2) of capillary blood hemoglobin and heart rate (HR) were evaluated in each patient using a portable pulse oximeter (Nellcor™ Portable SpO2 Patient Monitoring System PM1 0 N, Medtronic Inc., Dublin, Ireland). To evaluate the quality of life, it was used the Short Form-12 instrument. SF-12 is one of the generic instruments that has been widely used to measure quality of life. Each component is scored on a scale from 0 to 100 so higher scores represent better health. The PCS12 focuses on participants' general overall health, limitations in mobility, work, and other physical activities as well as limitations because of pain. The MCS12 includes participants' limitations in social activity, emotional state, and level of distraction (Corey J. H, 2017). The VAS is used to measure the pain. It is one of the most commonly scale used for assessment of the subjective perceived pain, indicated as numerical number or as a visual level on a predefined scale (Mathias H., 2006; Litcher-Kelly L., 2007)

Statistical Evaluation

The statistical analysis has been performed using the NCSS© for Windows Statistical Software package (NCSS© LLC, version 9, Kaysville, UT). Results were compared by Wilcoxon's signed rank test (paired sample) and by Wilcoxon's Rank Sum Test (two sample). A *p* value less than 0.05 was considered significant.

Intervention protocol

The aim of early mobilization in the post-operative period is to mitigate the muscle loss, impaired pulmonary function and thromboembolic complications associated with bed rest (Winkelman C., 2007). Indeed, patients should be encouraged to spend at least two hours out of bed on the day of surgery and six hours per day until discharge (Lassen K, 2009). In both groups, mobilization was encouraged early after the operation. From the first postoperative day to the last postoperative day, patients were encouraged as soon as possible to sit and stand out of bed and then walk. Patents were stimulated to walk at least one circuit of the ward (approximately 50 meters) and increase it up to five times. Finally, they should use regular incentive spirometry. Participants assigned to the EG received the Manual Diaphragm Release Technique associate with Yogic Breathing. The technique was performed with the patient in the supine position and the therapist

Table 3 Evaluation of pulmonary function in the EG from T0 (baseline) to T1 (after surgery)

Variabile	Count	Mean	Standard deviation	95,0% LCL of Mean	95,0% UCL of Mean	*p* value
FEV1 (L) T0 **FEV1 (L) T1**	20 20	2,4 2,2	0,9 0,9	2,0 1,7	2,9 2,6	0.0006
FVC (L) T0 **FVC (L) T1**	20 20	3,2 2,8	1,1 1,2	2,6 2,2	3,7 3,3	0,000 8
PEF (L/s) T0 **PEF (L/s) T1**	20 20	5,5 4,7	2,2 2,1	4,5 3,7	6,6 5,7	0,0007
FEV1 T0 **FEV1 T1** **(% predicted value)**	20 20	98,4 85,4	24,4 22,9	87,0 74,7	109,8 96,1	0.0003
FVC T0 **FVC T1** **(% predicted value)**	20 20	100,5 86	26,1 24,4	88,2 74,5	112,8 97,4	0,0004
PEF T0 **PEF T1** **(% predicted value)**	20 20	80,6 67,5	22,1 20,9	70,2 57,7	91,0 77,3	0,001

Abbreviations: FVC forced vital capacity, FEV1 forced expiratory volumes 1 s, PEF peak expiratory flow.
Wilcoxon's signed rank test (paired sample)

standing above the patient's head. Then, the therapist made manual contact with the fingers bilaterally to the underside the costal cartilages which connect the upper ten pairs of ribs to the sternum. The lowest four costal cartilages, the seventh, eighth, ninth, and tenth, join on to one another in series, forming the costal arch. Subsequently, the patients were asked to inhale by first expanding the abdomen and the chest using one slow and uninterrupted movement, followed by a retention and then a passively exhalation. The timeline breathing pattern was as follows: 4 seconds (s) of inspiration, 4 s of air retention, and 8 s of expiration. In the inspiratory phase, the therapist with your hands and fingers gently drives the elevation of the ribs. During exhalation the physiotherapist maintains this traction without increasing it, and so on, until the perception of no longer being able to pull the tissues. The technique associated with Yogic Breathing was performed in two sets of 10 yogic breaths, with a 30 s of interval between them (Barassi G., 2018; Rocha T, 2015).

■ RESULTS

The comparison of the significance of differences from T0 (baseline) to T1 (after surgery) in the EG (table 3) and SG (table 4) showed in both group a significant decrement of the pulmonary functions. Only the parameter FEV1 / FVC was not statistically significant.

The reduction of these values is due to the pain after surgery which is detrimental to the pulmonary movements. Indeed, the incision of the abdomen after

Table 4 Evaluation of pulmonary function in the SG from T0 (baseline) to T1 (after surgery)

Variabile	Count	Mean	Standard deviation	95,0% LCL of Mean	95,0% UCL of Mean	*p* value
FEV1 (L) T0 **FEV1 (L) T1**	20 20	2,3 1,8	0,9 0,8	1,8 1,4	2,7 2,2	0.00009
FVC (L) T0 **FVC (L) T1**	20 20	2,9 2,4	1,0 0,9	2,4 1,9	3,4 2,8	0,0001
PEF (L/s) T0 **PEF (L/s) T1**	20 20	4,9 3,3	1,9 1,5	4,0 2,6	5,9 4,0	0,0001
FEV1 % T0 **FEV1 % T1** **(% predicted value)**	20 20	86,9 70,1	21,1 22,8	77,0 59,4	96,8 80,8	0.00009
FVC % T0 **FVC % T1** **(% predicted value)**	20 20	87,5 72,5	22,2 21,2	77,0 62,6	97,9 82,4	0,00009
PEF % T0 **PEF % T1** **(% predicted value)**	20 20	72,2 47,7	21,5 16,6	62,1 39,9	82,3 55,5	0,0001

Abbreviations: FVC forced vital capacity, FEV1 forced expiratory volumes 1 s, PEF peak expiratory flow.
Wilcoxon's signed rank test (paired sample)

Table 5 Evaluation of outcome measures in the EG from T0 (baseline) to T1 (after surgery)

Variabile	Count	Mean	Standard deviation	95,0% LCL of Mean	95,0% UCL of Mean	*p* value
HR (bpm) T0 HR (bpm) T1	20 20	67,7 67,0	12,5 6,6	61,8 63,9	73,6 70,1	ns
SpO_2 %T0 SpO_2 %T1	20 20	97,9 99,1	1,2 1,0	97,3 98,6	98,5 99,5	0,010
MCS12 T0 MCS12 T1	20 20	47,7 55,1	12,2 9,0	42,0 50,8	53,4 59,3	0,011
PCS12 T0 PCS12 TI	20 20	43,1 34,0	12,4 7,2	37,3 30,7	48,9 37,4	0,003
VAS T0 VAS T1	20 20	1,4 2,5	2,4 1,0	0,2 2,0	2,5 2,9	0,021
ADL T0 ADL T1	20 20	5,8 5,9	0,3 0,2	5,6 5,8	6,0 6,0	ns

Abbreviations: HR heart rate, SpO2 oxygen saturation, MCS12 Mental Component Summary (SF-12), PCS12 and Physical Component Summary (SF-12), VAS Visual Analogue Scale.
Wilcoxon's Rank Sum Test (paired sample)

surgery, the pain and the medication were definitely uncomfortable when the patients was performing the spirometry test. Furthermore, the movements of the diaphragm and the intra-abdominal pressure during the execution of the test was perceived by the patient on the sutures of the surgical wound. In both groups there was a not significant reduction of HR and a statistically significant increment of SpO2 in the EG which is probably linked with a more effective oxygenation and pulmonary functionality after surgery compared with SG. In both groups we recorded in the SF-12 scale a statistically significant increment in the MCS-12. Conversely, it was observed a reduction of the PCS-12 index maybe due to the surgery wound, drains and the bladder catheter. Besides, the VAS score confirmed the feeling of discomfort of the patients after surgery with a statistically significant increment of its values in each group. Finally, ADL score demonstrated that early mobilization could promote the conservation of the basic autonomy, after the operation (table 5, table 6).

So, this is a crucial parameter before the discharge. Comparison of the measurements of both group at T1 showed a significant difference in PEF parameter. In the EG we recorded a higher value than SG maybe due to a better compliance of the pulmonary functions and diaphragm's movements in that group. Furthermore, there was a significant different in FEV1 and SpO2 parameters that confirm the better managing of breathing in EG. Finally, the VAS scale displayed a significant increment at T1 more in SG. That probably was linked with a less subjective perception of

Table 6 Evaluation of outcome measures in the SG from T0 (baseline) to T1 (after surgery)

Variabile	Count	Mean	Standard deviation	95,0% LCL of Mean	95,0% UCL of Mean	*p* value
HR (bpm)T0 HR (bpm) T1	20 20	69,0 73,7	12,6 13,5	63,1 67,3	74,9 80,0	ns
SpO_2% T0 SpO_2 % T1	20 20	97,8 97,5	1,3 1,8	97,1 96,6	98,4 98,3	ns
MCS12 T0 MCS12 T1	20 20	46,3 52,9	12,1 10,3	40,6 48,1	52,0 57,8	0,019
PCS12 T0 PCS12 TI	20 20	46,1 33,9	9,7 9,4	41,5 29,5	50,7 38,4	0,0005
VAS T0 VAS T1	20 20	2,8 4,5	2,7 1,9	1,5 3,5	4,0 5,4	0,016
ADL T0 ADL T1	20 20	5,6 5,5	1,3 1,3	4,9 4,8	6,2 6,1	ns

Abbreviations: HR heart rate, SpO2 oxygen saturation, MCS12 Mental Component Summary (SF-12), PCS12 and Physical Component Summary (SF-12), VAS Visual Analogue Scale.
Wilcoxon's Rank Sum Test (paired sample)

Table 7 Comparison of results of outcome measures in the EG and SG from T0 (baseline) to T1 (after surgery)

Variabile	Count	Mean	Standard deviation	95,0% LCL of Mean	95,0% UCL of Mean	*p* value
FEV1 (L) T0 FEV1 (L) T1	20 20	2,2 1,8	0,9 0,8	1,7 1,4	2,6 2,2	ns
FVC (L) T0 FVC (L) T1	20 20	2,8 2,4	1,2 0,9	2,2 1,9	3,3 2,8	ns
PEF (L/s) T0 PEF (L/s) T1	20 20	4,7 3,3	2,1 1,5	3,7 2,6	5,7 4,0	0,036
FEV1 % T0 FEV1 % T1 (% predicted value)	20 20	85,4 70,1	22,9 22,8	74,7 59,4	96,1 80,8	0,016
FVC % T0 FVC % T1 (% predicted value)	20 20	86 72,5	24,4 21,2	74,5 62,6	97,4 82,4	ns
PEF % T0 PEF % T1 (% predicted value)	20 20	67,5 47,7	20,9 16,6	57,7 39,9	77,3 55,5	0,002
HR (bpm) T0 HR (bpm) T1	20 20	67,0 73,7	6,6 13,5	63,9 67,3	70,1 80,0	ns
SpO_2 %T0 SpO_2 % T1	20 20	99,1 97,5	1,0 1,8	98,6 96,6	99,5 98,3	0,0008
MCS12 T0 MCS12 T1	20 20	55,1 52,9	9,0 10,3	50,8 48,1	59,3 57,8	ns
PCS12 T0 PCS12 TI	20 20	34,0 33,9	7,2 9,4	30,7 29,5	37,4 38,4	ns
VAS T0 VAS T1	20 20	2,5 4,5	1,0 1,9	2,0 3,5	2,9 5,4	0,001
ADL T0 ADL T1	20 20	5,9 5,5	0,2 1,3	5,8 4,8	6,0 6,1	ns

Abbreviations: FVC forced vital capacity, FEV1 forced expiratory volumes 1 s, PEF peak expiratory flow, HR heart rate, SpO2 oxygen saturation, MCS12 Mental Component Summary (SF-12), PCS12 and Physical Component Summary (SF-12), VAS Visual Analogue Scale. Wilcoxon's Rank Sum Test (two sample)

the pain in the EG than SG (table 7).

In addition, the post-operative setting showed that the EG was in a favorable clinical condition than the SG. Indeed, the latter was hospitalized for a longer period than EG and received more ventilation hours. Moreover, in EG the number of patients that had postoperative complications was less than SG (table 8).

DISCUSSION

In comparison to other surgery approaches, colorectal surgery is associated with a high risk of morbidity and mortality (Sarah E., 2016). Postoperative patients experience a surgical stress response that affects several physiological and physical response (Holte K, 2002). By reducing physiological and psychological stress associated with operations and minimizing pain and discomfort (Kehlet H,2005), early postoperative mobilization is a crucial principle of good physiotherapy practice. It can accelerate the achievement of discharge criteria, and it can reduce the rate of postoperative pulmonary complications, venous thromboembolism and infection associated with bed rest (Epstein NE., 2014; Winkelman C,2007). Furthermore, early mobilization can improve pulmonary functions and arterial oxygenation more than breathing exercises alone (Zafiropoulos B., 2004). Indeed, numerous clin-

Table 8 Patient's Characteristics in the post-operative setting

Group		SC	EG
Number of patients that had post-op pulmonary complications, n (%)		2 (10%)	1 (5%)
ICU hours, mean (SD)		60 ±30	39 ±27
Days in hospital, mean (SD)		6,8±2	7±2
Type of surgery, n (%)	Right hemicolectomy	12(60%)	8(40%)
	Left hemicolectomy	6(30%)	9(45%)
	Anterior resection	2(10%)	3(15%)
Technique of surgery, n (%)	Laparotomy	3(15%)	2(10%)
	Laparoscopy	13(65%)	14(65%)
	Robotic surgery	4(20%)	4(20%)

ical trials have provided positive evidence of benefits of utilizing fast-track programs for surgical patients, including accelerating the return of gastrointestinal function, and reducing morbidity and mortality rates (Khoo CK, 2007; Dongjie Y, 2012). In addition, the effects of Yoga have been explored in a large number of patient populations, as well as in healthy individuals. In recent years, it has begun to examine the effects of Yoga in cancer patients and individuals who have completed cancer treatment. Results from this literature suggest that yoga is a feasible intervention for a wide range of cancer patients and survivors with positive effects on a variety of outcomes, including sleep quality, cancer-related distress, cancer related symptoms and quality of life (Bower J. E, 2005). Pranayama or Yogic Breathing is a kind of breath regulation which is considered as an essential component of Yoga (A. A. Saoji,2019). Pranayamic breathing, defined as a manipulation of breath movement, can contribute to a physiologic response such as a reduction of: oxygen consumption, heart rate and blood pressure. Besides, it can increase parasympathetic activity accompanied by the experience of alertness and reinvigoration but, this mechanism of how pranayamic breathing interacts with the nervous system remains to be clearly understood (Ravinder J,2006). Pranayama is a type of yogic breathing exercise. Regular, slow and forceful inspiration and expiration for a longer duration during the pranayama practice, leading to strengthening of the respiratory muscles. Pranayama training manages improvement in the expiratory power and decreases the resistance to the air flow in the lungs. Moreover, it increases in the voluntary breath holding time. This could be due to acclimatization of the chemoreceptors to hypercapnoea (Shankarappa V., 2012). Moreover, to enhance pulmonary movements, some evidences suggested that manual therapy has the potential to affect respiratory mechanics in chronic pulmonary diseases, which includes an increase in flexibility of the chest wall and thoracic excursion and indirectly an improvement in exercise capacity and lung function (S. E. Bockenhauer, 2002; R. Engel, 2011). The diaphragm, which is the main inspiratory muscle, generates a craniocaudal movement during its contraction (W. D. Reid, 1995). The Manual Diaphragm Release Technique is an intervention intended to relax the diaphragm by enhancing its contraction and relaxation, chest wall mobility and for this reason creating a greater pressure gradient between the thorax and abdomen (L. Chaitow, 2002; F. J. Gonz´alez-Alvarez, 2016). During Manual Diaphragm Release Technique, the positive effects on diaphragmatic mobility may be due to the traction of the lower rib cage in a cranial direction and manual compression in the area of insertion of the anterior costal diaphragm fibers by the manual action on the underside of the last four costal cartilages which allows lengthening the diaphragm in its insertional zone (T.Rocha,2015). Yelvar YDG evaluated beneficial effects of manual therapy on inspiratory muscle strength and respiratory functions in patients with COPD (G.D. Yilmaz, 2016). An interesting study evaluated the effect of diaphragm as well as costal manipulation on functional capacity and pulmonary function in patients with moderate COPD. The result showed that both techniques were effective tools in improving pulmonary function and functional capacity (A. A. Abdelaal 2015). However, there is a lack of evidence regarding comparison of Diaphragmatic stretching technique and Manual Diaphragm Release technique on diaphragmatic excursion in patients with COPD (Aishwarya N., 2019). In this research, we hypnotized that manipulation of breath movement may be crucial in patients underwent colorectal resection for reducing postoperative pulmonary complication and restoring normal pulmonary functions. Additionally, Manual Diaphragm Release Technique may emphasize diaphragm's movements and relaxation thereby enhancing pulmonary functions and wellbeing in patient after surgery. Our result suggest that this approach could be a valid tool in that type of patients' population.

CONCLUSIONS

This study demonstrated that there is the place for cooperation between yogic breathing and Manual Diaphragm Release Technique after colorectal surgery. The comparison between the groups showed that the SG has been in a favorable clinical condition to prevent PPCs than the CG group. Thus, this new approach could be used as a component of the management of patients who have undergone colorectal surgery for cancer. Future, larger studies will need to confirm this finding and to assess the effect of early mobilization added to a Yogic Breathing and Manual Diaphragm Release Technique in patients underwent colorectal resection.

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Competing Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author's contributions

G.B. designed and directed the project and performed the experiments; A.D.I. and E.M. analyzed spectra and wrote the article.

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