

High, Low, and Minimal Flow Anaesthesia Management: Effects on Oxygen Reserve Index and Arterial Partial Oxygen Pressure

Ramazan Dastan¹, Hale Kefeli Celik¹ and Zahide Doganay²

¹Department of Anaesthesiology and Reanimation, Samsun Education and Research Hospital, Samsun, Turkey

²Department of Anaesthesiology and Reanimation, Medical School, Kastamonu University, Kastamonu, Turkey

ABSTRACT

Objective: To determine the oxygen reserve index (ORI) as a supporting parameter to the arterial partial oxygen pressure (PaO₂) in blood gases in hypoxia and hyperoxia monitoring with different fresh gas flows (FGF) in patients undergoing abdominal surgery.

Study Design: Randomised controlled trial.

Place and Duration of the Study: Department of Anaesthesiology and Reanimation, Samsun Education and Research Hospital, Turkey, from January to September 2020.

Methodology: The study population of ninety patients was divided into three groups. After the high-flow period, the inspired oxygen fraction (FiO₂) and flow-guided ventilation (FGF) were set to be 4 L/m and 40% in Group H (high-flow), 1 L/m and 50% in Group L (low-flow), and 0.5 L/m and 68% in Group M (minimal-flow), respectively.

Results: There was a very high statistically positive correlation between PaO₂ and ORI in H, L, and M groups. When using a cut-off value of 0.005 for ORI for the detection of PaO₂ >100 mmHg, the area under the curve (AUC) was 0.97 (p<0.001) with a sensitivity of 94.4% and specificity of 95.3%. The AUC was detected to be 0.95 in receiver operating characteristic (ROC) analysis when the hyperoxia cut-off value of ORI was used to determine PaO₂ >150 mmHg in the estimation of hyperoxia.

Conclusion: ORI can be used to complement SpO₂ in low-flow anaesthesia in patients undergoing abdominal surgeries, provide guidance for PaO₂, give information about tissue oxygen delivery, and contribute to the individualisation of oxygen therapy, and will therefore be included in the standard monitoring in the future.

Key Words: Anaesthesia, Index, Inhalation, Oxygen, Pressure, Surgery.

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INTRODUCTION

Maintenance of oxygen distribution at a level adequate to meet all metabolic needs is the primary goal of haemodynamic therapy during the anaesthesia of patients undergoing surgery. Low-flow anaesthesia (LFA) applications have benefits such as the reduction of cost, prevention of environmental pollution, preservation of moisture in inhaled gases, and prevention of microatelectasis formation. Therefore, interest in LFA applications has been increasing in the recent years.¹

Potential leaks, large system volume, inconsistency between the transferred and delivered fraction of inhaled gases, and accumulation risk of toxic compounds as well as hypoventilation are the main issues associated with LFA.²

Therefore, additional monitoring is needed for the safe application of low-flow techniques. Exhaled gas volume, airway pressure, the fraction of inspired oxygen (FiO₂), volatile anaesthetic agent concentration, carbon dioxide concentration, and peripheral oxygen saturation (SpO₂) values in anaesthesia applications must be constantly monitored within the scope of the Common European Standard (EN 740).³

The oxygen reserve index (ORI), derived from non-invasive multi-wavelength pulse co-oximeter measurements, has recently been defined as a new relative indicator of partial pressure of oxygen (PaO₂).⁴ ORI is an index between 0.0 and 1.0 and is reported when arterial oxygen saturation (SpO₂) is above 98%. Though initially developed to be sensitive for PaO₂ changes between 100-200 mmHg, ORI can also show PaO₂ changes when SpO₂ is above 98%.⁵ PaO₂ and SaO₂ do not demonstrate a linear relationship as SaO₂ rapidly decreases when PaO₂ falls below 70 mmHg. Therefore, ORI is an important parameter, as it can show the early change in PaO₂ before any change in SpO₂ occurs.^{6,7}

This study's objective was to investigate the use of continuous ORI monitoring as a backup to PaO₂ in blood gases during moni-

Correspondence to: Dr. Hale Kefeli Celik, Department of Anaesthesiology and Reanimation, Samsun Education and Research Hospital, Samsun, Turkey
E-mail: ck_hale@hotmail.com

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toring of hypoxia and hyperoxia when different flows of fresh gas are used in general anaesthesia.

METHODOLOGY

This study was carried out in accordance with the Declaration of Helsinki, approved by the Clinical Trials Ethics Committee of Samsun Education and Research Hospital (approval no. SBUSEAH-KAEK-2020/1), and registered on Clinicaltrials.gov with registration no: NCT05329233.

Between January and September 2020, this single-blind, randomised controlled trial enrolled 90 ASA II and III patients aged 18-75 years old who were due to undergo elective open abdominal surgery lasting <60 minutes under general anaesthesia. Closed envelope technique was used to randomise patients into three groups of Group M (minimal-flow), Group L (low-flow), and Group H (high-flow), each consisting of 30 patients. Envelopes were prepared and each patient randomly chose an envelope which included the name of the group they would be assigned to. Exclusion criteria was patients who did not give consent for inclusion in the study, those with finger deformities or low-blood flow that were unable to use the sensor, those with malignant hyperthermia and severe anaemia (haemoglobin <8 g/dL), who had alcohol or drug addiction, morbid obesity (BMI >40 kg/m²), history of chronic obstructive pulmonary disease, decompensated diabetes mellitus, severe cardiac, renal or hepatic insufficiency or cerebrovascular disease, and those with local anaesthetic or opioid sensitivity.

Patients were transferred into the operating room and anaesthesia was induced after preoxygenation (100% O₂, 6 L/min, 3 minutes). Patients were ventilated using a volume-controlled mode (Dräger Perseus® A500 Anaesthesia Workstation, Dräger, Germany) that allows for real-time monitoring of airway pressure, exhaled gas volume, FiO₂, volatile anaesthetic substance concentration, and carbon dioxide concentration according to the requirements of Common European Standard EN 740. The carrier gas was medical air. After intubation, end-tidal carbon dioxide (EtCO₂) was continuously measured, and tidal volume and ventilation rates were modified as needed to keep EtCO₂ between 30 and 40 mmHg. Patients in all three groups received continuous inhalational anaesthesia consisting of 0.05-0.2 mcg/kg/min of remifentanyl and FGF 4 L/min of a 50% oxygen-medical air mixture with 6-8% desflurane for maintenance. In all three groups, after intubation, a fresh gas flow of 4 L/min was used to administer 6% desflurane for 10 minutes, with the minimum alveolar concentration (MAC) value set at 1.

Subsequently, patients in Group H received 1 L/min of oxygen and 3 L/min of medical air (FGF 4 L/min, FiO₂ 40%); those in Group L received 0.37 L/min of oxygen and 0.63 L/min of medical air (FGF 1 L/min, FiO₂ 50%); and those in Group M received 0.3 L/min of oxygen and 0.2 L/min of medical air (FGF 0.5 L/min, FiO₂ 68%). In Group H, once an adequate MAC value

was reached 10 minutes after intubation, FiO₂ was lowered to 40% while fresh gas flow (FGF) was maintained at a constant rate of 1 L/min; in Group L and M, FGF was reduced to 1 L/min and 0.5 L/min, respectively, and the desflurane scale setting was adjusted to achieve MAC 1 for the remainder of the surgery.

Using the Masimo Radical 7 pulse CO-Oximeter, readings from a disposable, light-protected sensor (RD Rainbow Lite Set ORI Probe®, Masimo Corp., Irvine, CA) on the index finger of the left hand were recorded (Masimo Corp., Irvine, CA).

All patients had their vaporisers turned off at the conclusion of the operation, and high FGF (4 L/min, FiO₂ 100%) was applied to facilitate rapid extubation of the lungs following the administration of anaesthetic gases. In patients who did not develop complications during surgery, extubation was performed once spontaneous breathing had resumed and Sugammadex (2-4 mg/kg IV) had been given to reverse the residual muscle relaxation.

Demographic data of the patients such as age, gender, body weight, height, and body mass index were recorded before the operation. Nine different time points were used to record the parameters: T1 (5 minutes prior to induction of anaesthesia), T2 (following intubation), T3 (beginning of 0.5-1-4 L/min), T4 (10 minutes into low-flow), T5 (30 minutes into low-flow), T6 (60 minutes into low-flow), T7 (90 minutes into low-flow), T8 (120 minutes into low-flow), T9 (end of surgery, ventilation with 100% oxygen), and T10 (5 minutes after extubation).

According to the power and sample size test based on the previous studies in this field, the sample size alpha set at 0.01 and power of the test accepted as 0.95, it was calculated that a minimum of 27 people per group should be studied.^{8,9} In order to account for any possible patient dropouts, each of the groups was made with at least 30 participants that amounted to 90 in total.

Statistical tests were run on the SPSS Windows version 23.0 package software (Chicago, IL, USA), and results were considered significant when the p-value was <0.05. Normal distribution was analysed with Kolmogorov Smirnov and Shapiro-Wilk tests. In order to compare categorical data between different groups, a Chi-square test was conducted. Quantitative data with a normal distribution were compared between groups using one-way analysis of variance (ANOVA), while non-normal data were compared using the Kruskal-Wallis test. Repetitive analysis of variance was used for normally distributed data and Friedman test was used for data that were not normally distributed in examining the changes within groups according to the time. Spearman's rho correlation coefficient was used to examine the relationship between quantitative data that were not normally distributed. Linear regression was used to examine the impact of the PaO₂ parameter on the ORI. For quantitative data, analysis results were displayed as mean standard deviation and median (minimum-maximum), and for categorical data, results were displayed as frequency (percent).

Table I: Comparison of demographic data, ASA, anaesthesia, and surgical times between groups.

	Group H Median (min-max)/n(%)	Group L Median (min-max)/n(%)	Group M Median (min-max)/n(%)	Test statistic	p
Age (years)	65 (26 - 75)	59.5 (33 - 74)	63.5 (27 - 75)	$\chi^2 = 3.069^1$	0.216
Height (cm)	170 (150 - 190)	165 (152 - 182)	170 (155 - 180)	$\chi^2 = 1.664^1$	0.435
Weight (kg)	75 (54 - 89)	71 (40 - 110)	71 (60 - 94)	$\chi^2 = 1.095^1$	0.578
BMI (kg/m ²)	25.7 ± 2.3	25.6 ± 4.2	26.2 ± 3.6	F = 0.242 ¹	0.786
Gender (F/M)	7(23%)/23(77%)	6(20%)/24(80%)	8(27%)/22(83%)	$\chi^2 = 0.373^2$	0.830
ASA (II/III)	16(53%)/14(47%)	17(57%)/13(43%)	15(50%)/15(50%)	$\chi^2 = 0.268^2$	0.875
Anaesthesia time (min)	140 (110 - 150)	140 (110 - 155)	140 (100 - 150)	$\chi^2 = 0.339^1$	0.844
Operation time (min)	130 (90 - 145)	125 (90 - 145)	130 (90 - 135)	$\chi^2 = 0.651^1$	0.722

ASA: American Society of Anaesthesiologists, BMI: Body mass index; Min: minute. χ^2 = Kruskal-Wallis test statistic¹, χ^2 = Chi-square test statistic², F: One-way analysis of variance test statistic.

Table II: Examination of ORI and PaO₂ relations by time.

Time	Group H		Group L		Group M		Total	
	r	p	r	p	r	p	r	p
T1 ORI-PaO ₂	-0.026	0.891	0.398	0.030	0.316	0.089	0.215	0.042
T2 ORI-PaO ₂	0.524	0.003	0.318	0.086	0.416	0.022	0.426	<0.001
T3 ORI-PaO ₂	0.743	<0.001	0.541	0.002	0.383	0.037	0.573	<0.001
T4 ORI-PaO ₂	0.857	<0.001	0.744	<0.001	0.887	<0.001	0.841	<0.001
T5 ORI-PaO ₂	0.686	<0.001	0.837	<0.001	0.896	<0.001	0.793	<0.001
T6 ORI-PaO ₂	0.711	<0.001	0.732	<0.001	0.822	<0.001	0.759	<0.001
T7 ORI-PaO ₂	0.769	<0.001	0.765	<0.001	0.870	<0.001	0.808	<0.001
T8 ORI-PaO ₂	0.570	0.004	0.668	0.001	0.892	<0.001	0.761	<0.001
T9 ORI-PaO ₂	-0.024	0.898	-0.366	0.046	0.042	0.826	-0.131	0.219
T10 ORI-PaO ₂	0.468	0.009	0.202	0.286	0.316	0.089	0.363	<0.001

r: Spearman's rho correlation coefficient.

Table III: Results of regression analysis according to the groups.

Group	Independent variable	Beta	Standardised beta (95% CI)	p	F	p	r ²	Corrected r ²
H	Constant	-0.528	(-0.602 -0.455)	<0.001	552.494	<0.001	0.654	0.653
	PaO ₂	0.006	0.809 (0.005 - 0.006)	<0.001				
L	Constant	-0.503	(-0.575 -0.43)	<0.001	535.826	<0.001	0.649	0.648
	PaO ₂	0.006	0.806 (0.005 - 0.006)	<0.001				
M	Constant	-0.581	(-0.651 -0.511)	<0.001	675.286	<0.001	0.698	0.697
	PaO ₂	0.006	0.836 (0.006 - 0.007)	<0.001				
Total	Constant	-0.534	(-0.575 -0.492)	<0.001	1740.142	<0.001	0.665	0.664
	PaO ₂	0.006	0.815 (0.006 - 0.006)	<0.001				

In all groups, an increase of 1 unit for PaO₂ led to 0.006 unit increase in ORI.

RESULTS

Demographic characteristics of patients, ASA physical risk classification, anaesthesia and surgery durations were similar between groups (p>0.05, Table I).

There was no statistically significant difference between the distributions of heart rate, systolic blood pressure, and mean blood pressure values measured at different times between groups (p>0.05). Mean SpO₂ at T4, T6, and T7 differed significantly between groups (p=0.009). This difference is due to the difference between Group M and H.

There was a very high positive correlation between ORI and PaO₂ at T4 in Group H (p<0.001; r=0.857). A very high statistically significant positive correlation was found between ORI and PaO₂ in Group L (p<0.001; r=0.837) and in Group M (p<0.001; r=0.896) at T5 and between ORI and PaO₂ at T4 regardless of the group (p<0.001; r=0.841, Table II).

Regression models were established for each group and regardless of the groups, they were found to be statistically

significant (p<0.001). In all groups, an increase of 1 unit for PaO₂ led to 0.006 unit increase in ORI (Table III).

When using a cut-off value of 0.005 for ORI for the detection of PaO₂ >100 mmHg, the area under the curve (AUC) was calculated to be 0.97 (p<0.001) with a sensitivity of 94.4% and specificity of 95.3%. Similarly, when using a cut-off value of 0.295 for ORI for the detection of PaO₂ >150 mmHg, the area under the curve (AUC) was calculated to be 0.95 (p<0.001) with a sensitivity of 87.7% and specificity of 87.6%.

When ORI >0.005, 99% of PaO₂ values were observed as being >100 mmHg, and when ORI <0.005, 80% of PaO₂ values were observed as being <100 mmHg. When PaO₂>100 mmHg, 95% of ORI values were found to be >0.005. When PaO₂<100 mmHg, 80% of ORI values were found to be <0.005 (Figure 1). When ORI >0.29, 80% of PaO₂ values were observed to be >150 mmHg. When ORI <0.29, 92% of PaO₂ values were observed to be <150 mmHg (Figure 2).

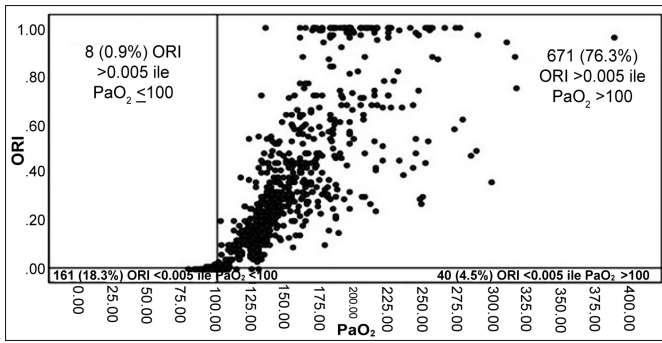


Figure 1: PaO₂ >100 mmHg and ORI ≥0.005 corresponding data.

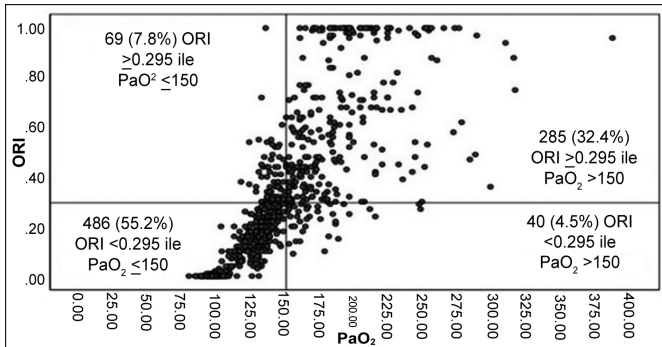


Figure 2: PaO₂ >150 mmHg and ORI ≥0.295 corresponding data.

DISCUSSION

Today, the most important factor preventing the routine use of the LFA technique is the possibility of hypoxia in patients. The clinicians are reluctant to use LFA as this is the most common reason cited in the studies.¹⁻³ In the study evaluating the effect of anaesthesia applications with different FGF on oxygenation in patients undergoing abdominal surgery, it was aimed to show that ORI monitoring is a valuable parameter in demonstrating hypoxia and hyperoxia, since ORI was positively correlated with PaO₂ in blood gases.

In order to provide adequate oxygen support and prevent hypoxia in anaesthesia applications that use reduced fresh gas flow, the inspiratory oxygen concentration should be adjusted to at least 40%, because inspiratory air also contains expiratory air with low oxygen concentrations.¹⁰ As the rebreathing rate increases even more in minimal-flow anaesthesia, when FGF is reduced, the oxygen concentration in the fresh gas should be increased to the minimum value of 50% or even 60% to prevent hypoxic gas mixture.¹¹ Based on these findings, the patients were divided into three categories for examination. After the initial high-flow period, the FGF and FiO₂ were lowered to 4 L/m and 40%, 1 L/m and 50%, and 0.5 L/m and 68%, in Group H, L, and M, respectively. Çolak *et al.* conducted a study comparing two different FGFs (Group 10: 10 ml/kg and Group 20: 20 ml/kg) to determine the feasibility, safety, and economic outcomes of low-flow anaesthesia by body weight.¹² The authors reported that 61% (min. 56%, max. 67%) oxygen concentration in Group 10 and 47% (min. 43%; max. 51%) oxygen concentration in Group 20 were necessary to maintain FiO₂:0.4, the

safe limit in FGF, and to provide adequate oxygenation during LFA in liver transplantation. Group 10 and 20 both showed steadily increasing ORI values. The values provided here are consistent with what is encountered in this study.

In patients undergoing oxygen therapy, SpO₂ is seriously limited for the evaluation of hypoxia or hyperoxia. The relationship between arterial oxygen saturation and partial oxygen pressure is expressed by the oxyhaemoglobin dissociation curve, which includes three different ranges (hypoxia, normoxia, and hyperoxia). When the relationship between SaO₂ and PaO₂ is evaluated, a well-defined sigmoidal curve is achieved until PaO₂ reaches approximately 80 mmHg or above, and the corresponding SaO₂ remains constant at 100%. However, at higher oxygen partial pressures, SaO₂ cannot increase any further. Thus, SpO₂ can no longer be used to assess the oxygen partial pressure because haemoglobin is completely saturated. Also, SpO₂ may not provide a preliminary warning of impending hypoxia in cases where PaO₂ drops, as SpO₂ may not decrease until PaO₂ drops below 80 mmHg.⁶ ABG analysis is considered the gold standard for monitoring tissue oxygenation status. However, it is costly, invasive, causes blood loss and is associated with delayed results, which are the weaknesses of this method.¹³ ORI, a current non-invasive monitoring method, has become clinically important due to its relative correlation with PaO₂.⁴

The ORI value can help us to detect impending desaturation before a change in saturation is noticed. In a study of paediatric patients, Szmuk *et al.* reported that ORI detected impending desaturation within a median value of 31.5 seconds (IQR 19 - 34.3 sec.) before a change in saturation was noted.¹⁴ This can provide clinicians with a critical window of opportunity to institute preventative measures.

In this study, when the preoperative and post-extubation time were included, SpO₂ varied between 95-100%; on the other hand, PaO₂ was observed in a wider range between 79-388 mmHg. Also, ORI varied between 0-1. ORI values were zero in 10, 11, and 12 patients in Group H, L, and M, respectively. Since ORI was being continuously monitored, it showed zero at recorded time intervals in 24 patients and at non-recorded time intervals in 9 patients. PaO₂ values ranged from 93-108 mmHg when ORI values were zero. When partial pressure of oxygen began to decrease, ORI decreased along with it. However, the SpO₂ only began to decrease once ORI reached zero, leaving far less time to recognise the problem and adequately address it. At the time of T9, when 100% oxygen was administered, SpO₂ showed 100% in the majority of patients, while ORI showed value of 1 in 70%. Under oxygen therapy, SpO₂ increased first, and when it reached 99 or 100%, ORI began to increase. ORI monitoring came to the fore especially as SpO₂ has limited capacity for evaluation and PaO₂ is invasive and its results are not real-time.

There is a very high statistically positive correlation ($p < 0.001$; $r = 0.913$) between PaO_2 and ORI, which is similar between Group H, L, and M, regardless of the group. In the linear regression analysis, the strongest relationship ($p < 0.001$; $r = 0.841$) was found between ORI and PaO_2 at the T4 time point (10th minute of low-flow), regardless of the group. It was observed that ORI showed zero in the minimal-flow group at most at the time of T4 (10th minute of low-flow). The fact that being susceptible to hypoxia in the first 10 minutes after FGF reduction and the strong correlation of ORI and PaO_2 in this interval makes ORI monitoring important as an early warning of hypoxia in low-flow anaesthesia.

The primary goal in the haemodynamic management of perioperative patients is to maintain oxygen delivery at a level adequate to meet all metabolic needs. Ehrenfeld *et al.*, in a retrospective review of 95,407 anaesthesia records from two different institutions, reported that 6.8% of the patients experienced a hypoxic ($\text{SpO}_2 < 90\%$) event during the intraoperative period, while 3.5% of the patients experienced a severe hypoxemic ($\text{SpO}_2 < 85\%$) event that lasted longer than 2 minutes.¹⁵ Therefore, ORI is important in terms of outcome, as it allows intervention when the patient is approaching hypoxia yet within safe limits before SpO_2 begins to decrease. In a recently performed study of health volunteers, the authors reported the cut-off value of ORI to be 0.01 (sensitivity 0.99, specificity 0.82) to detect $\text{PaO}_2 < 100$ mmHg.⁴ In this study, the ORI cut-off value was found to be ~0.01 to detect $\text{PaO}_2 < 100$ mmHg. The ORI cut-off value determined in this study can be considered as the safe limit, as it predicts hypoxia, and may contribute to the widespread use of low-flow anaesthesia.

Scheeren *et al.* reported mild hyperoxia as $\text{PaO}_2 < 150$ mmHg and moderate hyperoxia as $\text{PaO}_2 \geq 150$ mmHg in line with ORI and PaO_2 values. The authors reported $\text{PaO}_2 < 150$ mmHg and ORI 0.3 (sensitivity and specificity; $\geq 85\%$, $\geq 80\%$, respectively) at the limit of mild hyperoxia.¹⁶ Similarly, in this study, for hyperoxia cut-off value of the ORI determined to be used in the estimation of hyperoxia in cases with a PaO_2 value > 150 , AUC was found to be 0.95 in ROC analysis. At the value of 0.29, ORI sensitivity was calculated as 87.7% and specificity as 87.6%. ORI > 0.29 values may be a parameter in clinical use in estimating hyperoxia, owing to these high sensitivity and specificity values. Unnecessary hyperoxia can be avoided without the need for frequent arterial blood gas using the ORI cut-off value found in this study.

Applegate *et al.* investigated the relationship between 1594 ORI values obtained from 106 patients who underwent surgical intervention and PaO_2 values measured from 485 arterial blood gas analyses. When ORI > 0.24 , PaO_2 was ≥ 100 mmHg in all measurements, and when ORI > 0.55 , 96.6% of PaO_2 was determined to be > 150 mmHg. The authors reported that linear regression analysis of ORI and PaO_2 from 100 mmHg to 240 mmHg showed a positive correlation.⁵ In the

linear regression analysis conducted in this study, it was observed that when ORI > 0.005 , 99% of all PaO_2 values were > 100 mmHg, and when ORI > 0.29 , 80% of the PaO_2 values were > 150 mmHg. Each 0.06 unit increase in the ORI values measured, regardless of the groups, corresponded to a 10 mmHg ($p = 0.001$; $r = 0.665$) increase in PaO_2 . Due to this correlation, the ORI can also be used for FiO_2 titration during anaesthesia. These data demonstrated that ORI can be effectively used for monitoring in low-flow anaesthesia, and can be especially useful for the early warning of impending SpO_2 decrease.

The first limitation of this study was the duration (two hours) of the surgical procedures included in the study. In surgeries lasting longer than two hours, the results may be different. Secondly, the study was conducted in patients who underwent open surgery. Results may be different in patients who underwent laparoscopic surgery. Finally, the study could not be designed to be methodologically blind.

CONCLUSION

In major surgeries, LGA techniques can be an alternative to high-flow anaesthesia by monitoring tissue oxygen delivery parameters. The ORI values determined for PaO_2 values < 100 mmHg and > 150 mmHg constituted a safe limit in the range of 0.01 to 0.29 to protect against hyperoxia and hypoxia in anaesthesia. The strong correlation between ORI and PaO_2 can be utilised in LFA methods. The inclusion of ORI as standard monitoring in the future will be a useful complementary technique to SpO_2 in LFA applications in surgeries-providing guidance for PaO_2 , giving information about tissue oxygen delivery, and contributing to the individualisation of oxygen therapy and patient safety.

FINANCIAL DISCLOSURE:

This study was funded by the Interventional Clinical Research Ethics Committee of Samsun Research and Education Hospital, Turkey.

ETHICAL APPROVAL:

The present study was carried out in accordance with the Declaration of Helsinki and approved by Clinical Research Ethics Committee of Samsun Education and Research Hospital (approval no. SBUSEAH-KAEK-2020/1), and was also registered in the Clinicaltrials.gov clinical trials registry (no. NCT05329233).

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PATIENTS' CONSENT:

Written informed consents were taken from all patients included in the study.

COMPETING INTEREST:

The authors declared no conflict of interest with respect to the authorship and/or publication of this article.

AUTHORS' CONTRIBUTION:

RD, HKC: Conception and design, drafting of the manuscript, and material preparation.

RD: Data collection.

ZD: Supervision and manuscript editing.

All authors approved the final version of the manuscript to be published.

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