Paediatrica Indonesiana

p-ISSN 0030-9311; e-ISSN 2338-476X; Vol.62, No.2(2022). p.104-14; DOI: 10.14238/pi62.2.2022.104-14

Original Article

Resuscitation of very preterm infants with 30% *vs.* 50% oxygen: a randomized controlled trial

Risma Kerina Kaban[1], Asril Aminullah[1], Rinawati Rohsiswatmo[1], Badriul Hegar[1], Abdurahman Sukadi[2], Peter Graham Davis[3]

Abstract

Background Preterm infants are susceptible to the damaging effects of hyperoxia which may lead to bronchopulmonary dysplasia (BPD) and intestinal damage. Hyperoxia also affects intestinal microbiota. The optimal initial FiO_2 for the resuscitation of premature infants is unknown.

Objective To determine the effect of different initial oxygen concentrations on BPD, oxidative stress markers, damage to the gastrointestinal mucosa, and the intestinal microbiome.

Methods We conducted an unblinded, randomized controlled clinical trial in premature infants requiring supplemental oxygen in the first minutes of life. Infants started at an FiO_2 of either 30% (low) or 50% (moderate), which was adjusted to achieve target oxygen saturations (SpO_2) of 88-92% by 10 minutes of life using pulse oximetry. The primary outcome was incidence of BPD. Secondary outcomes included markers of oxidative stress [oxidized glutathione (GSH)/reduced glutathione (GSSG) ratio and malondialdehyde (MDA)], intestinal integrity indicated by fecal alpha-1 antitrypsin (AAT), and intestinal microbiota on fecal examination.

Results Eighty-four infants were recruited. There was no significant difference in rates of BPD between the 30% FiO_2 and 50% FiO_2 groups (42.8% *vs.* 40.5%, respectively). Nor were there significant differences in GSH/GSSG ratios, MDA concentrations, fecal AAT levels, or changes in facultative anaerobic and anaerobic microbiota between groups.

Conclusion In premature infants resuscitated using low *vs.* moderate initial FiO_2 levels, we find no significant differences in BPD incidence, markers of oxidative stress, intestinal mucosa integrity, or intestinal microbiota. **[Paediatr Indones. 2022;62:104-14 DOI: 10.14238/pi62.1.2022.104-14**].

Keywords: *bronchopulmonary dysplasia; hyperoxia; intestinal integrity and microbiota; oxidative stress; very premature infant*

Approximately 10% of all newborns require some assistance to establish breathing at birth, and approximately 1% need positive pressure ventilation.[1] Preterm infants are more likely to require help because they have reduced respiratory drive, and are more likely to be surfactant deficient.[2,3] Suboptimal resuscitation of premature infants may lead to death or long-term neurodevelopmental impairment.[4] Some premature infants may need supplemental oxygen during transition from intrauterine to extrauterine. Excessive oxygen concentrations may increase oxidative stress.[5,6] Premature infants with low antioxidant levels are less able to deal with the deleterious effects of reactive oxygen species (ROS), which may damage renal, retinal, pulmonary, and intestinal cells.[7-10] Oxidative stress is one of the major pathogenic mechanisms leading to preterm morbidities including bronchopulmonary

From the Department of Child Health, Universitas Indonesia Medical School/Dr. Cipto Mangunkusumo Hospital, Jakarta[1] and Universitas Padjadjaran Medical School/Dr. Hasan Sadikin Hospital, Bandung[2], Indonesia; Department of Newborn Research, Royal Women's Hospital, Parkville, Victoria, Australia.[3]

Corresponding author: Risma Kerina Kaban. Department of Child Health Universitas Indonesia Medical School. Kiara Building Level 11, Jl. Pangeran Diponegoro No.71, Senen, Central Jakarta, Special Capital Region of Jakarta 10320, Indonesia. Email: rismakk@yahoo.co.uk.

Submitted September 22, 20201 Accepted April 12, 2022.

dysplasia (BPD).[11-12]

Several studies have evaluated the relationship between initial FiO_2 in resuscitation and BPD.[5,6,13-14] It is likely that oxidative stress also affects intestinal permeability and microbiota. Intestinal inflammation and epithelial damage may lead to increased permeability of the intestinal wall, which can be detected using alpha-1 antitrypsin (AAT) in the stool.[15-17] Increased intestinal oxygen exposure may also change the balance of bacterial populations, since anaerobic microbes cannot survive in an oxygen-rich environment.[18-20] The optimal concentration of oxygen used during resuscitation of preterm newborns has not been well-established. We aimed to compare BPD incidence, intestinal integrity, markers of oxidative stress, and microbiota in premature infants who received initial resuscitation using low (30%) *vs.* moderate (50%) FiO_2.

Methods

This unblinded, controlled, randomized clinical trial in very premature infants who required supplemental oxygen in the first minutes of life was conducted at Bunda Menteng Hospital and Dr. Cipto Mangunkusumo Hospital, Jakarta, the central tertiary referral hospitals in Indonesia, between January and September 2015. The study was approved by the Universitas Indonesia Faculty of Medicine Ethics Committee. Consecutive sampling and block randomization were performed. The randomization sequence used random permuted blocks with 4 block sizes. The group allocation was placed in a sealed opaque envelope to be opened by the clinical team when the infant became eligible for study inclusion. Subjects were randomized into two study groups, the moderate group (initial resuscitation with 50% FiO_2) and the low group (initial resuscitation with 30% FiO_2). The interventions were performed by a high-risk resuscitation team. Pregnant women at risk of delivery between 25-32 weeks were approached antenatally for written informed consent.

The primary outcome was any BPD, as defined by Ehrenkranz *et al.*[21] The secondary outcomes were changes in the GSH/GSSG ratio, plasma serum MDA levels, fecal AAT levels, and the proportion of facultative anaerobic microorganisms (*Klebsiella pneumoniae* and Acinetobacter) as well as anaerobic microorganisms (Lactobacillus and Bifidobacteria).

The participants were infants between 25-32 weeks gestational age who required supplemental oxygen for any of the following reasons: (i) respiratory distress, (ii) bradycardia [heart rate <100 beats/minute (BPM)], or (iii) hypotonia and hyporeactivity. Gestational age was based on early ultrasound if available, or the first day of the last menstrual period. Exclusion criteria were uncertain gestational age and/or severe genetic or congenital defects affecting survival, such as trisomy 13 or 18, cyanotic congenital heart disease, or diaphragmatic hernia.

The resuscitation team for high-risk infants (25-32 weeks of gestation) was comprised of a neonatologist or pediatrician, residents, and nurses presented at the delivery. Monitoring of O_2 saturation was performed using a *Radical Oximeter* (*Massimo Co.*, Irvinne, CA) set to maximal sensitivity and two-second averaging.[5,13,14,22]

The saturation probe was applied to the right hand within 30 seconds after birth. Resuscitation was commenced using air. If supplemental oxygen or ventilation were required, a consecutively numbered, sealed, opaque envelope was opened by the resuscitation team and infants were randomized into either the moderate oxygen group (FiO_2 50%) or the low oxygen group (FiO_2 30%). The oxygen blender was set to provide the allocated FiO_2.

Resuscitation was performed according to the *2010 American Academy of Pediatrics (AAP) Guidelines*,[23] with slight modification of FiO_2 administration and saturation targets as explained below. All infants received resuscitation using the T-piece resuscitator (*Neopuff: Fisher & Paykel,* Auckland, New Zealand). The device was used to provide continuous positive airway pressure (CPAP) or intermittent positive pressure ventilation with end-expiratory pressure according to clinical need.

Immediately after birth, the resuscitation team put the infant under a radiant warmer, performed suctioning, applied an oximeter probe to the right wrist and connected it to the pulse oximeter.[8] A resident determined the initial heart rate by auscultation. When the SpO_2 and heart rate were available on the pulse oximeter, auscultation was ceased. If the heart rate was <100 BPM or if there was increased respiratory effort within 30 seconds after birth, the randomization envelope was opened and the assigned oxygen concentration delivery commenced. The infant

initially received 7 cm H_2O positive-end-expiratory pressure (PEEP) with a CPAP mask. If there was an inadequate clinical response (HR <100 BPM) after CPAP treatment for 30 seconds, the FiO_2 was increased in increments of 10% every 60 seconds and intermittent positive pressure ventilation (IPPV) was provided with peak inspiratory pressure (PIP) of 20 cm H_2O and PEEP of 5 cm H_2O for 2 minutes. If there was no improvement, the PIP was increased to achieve a satisfactory SpO_2 (88-92% at 10 minutes of age), and a stable heart rate (>100 BPM). If positive pressure ventilation (PPV) was required beyond two minutes, endotracheal intubation could be performed.

The target SpO_2 was 88-92% by 10 minutes after birth. If the heart rate was >100 BPM, the physician did not change the FiO_2. The physician could change the FiO_2 only if there was a persistent bradycardia or SpO_2 >92% (FiO_2 should be reduced). If the heart rate was ≤ 60 BPM for more than 30 seconds during the PPV, the FiO_2 was increased to 100% and cardiac compressions were commenced. Once stable, the infants were transferred to the NICU.

Immediately after delivery, a 5 mL blood specimen was drawn from the umbilical cord to measure GSH/GSSG ratio and MDA. The tests were repeated on day three of life on a venous blood specimen. The tests were performed at the *Biochemistry Laboratory,* Faculty of Medicine, Universitas Indonesia. A 300 mg stool specimen within the first 24-48 hours was taken for PCR examination of intestinal microbiota and subsequently, the stool was examined using ELISA techniques to measure the AAT level and absolute number of bacteria in the stool at the *Laboratory of Biomolecular Gastroenterology*, Department of Pediatrics, Faculty of Medicine, Universitas Indonesia. In the laboratory, the stool specimen was either directly treated for DNA extraction, frozen for later DNA extraction, or refrigerated until the desired sample size for laboratory examination was reached. Analysis of stool bacteria using real time-PCR consisted of 2 stages: isolation of bacterial DNA from the stool and evaluation of the bacteria present, including *Klebsiella pneumoniae*, Acinetobacter, Lactobacillus, and Bifidobacterium. The results were expressed in copy number /200 mg of stool for each microorganism. The stool AAT results were expressed in mg/dL. MDA measurement was performed by the *Wills ED* method.[24] Evaluation of lipid peroxidation in lipids and biological membranes[24] and measurement of GSH/GSSG concentration were performed using a laboratory kit and ELISA technique.[25]

Data were entered and analyzed using *SPSS v.20* software. Data with normal distribution were expressed as mean and standard deviation, whereas skewed data were expressed as median and range. Bivariate analysis was performed to identify the inter-variable correlation. Chi-square test was used to compare rates of BPD. Unpaired T-test with an alternative Mann-Whitney test was conducted to identify differences in MDA level, GSH/GSSG ratio, AAT level, and intestinal microbiota. The primary analysis was by intention-to-treat (including babies who died before 28 days of age and those whose families asked to be moved to another hospital). In order to detect a reduction in BPD rate from 45% to 15% with a statistical power of 80%, the minimum required sample size was 35 subjects per group. To allow for a 20% drop-out rate, 42 infants were recruited into each group.

Results

Of 114 infants born at 25-32 weeks gestational age, 30 infants were excluded because of parental consent refusal (6 infants), no respiratory distress (3 infants), congenital defect (4 infants), born before informed consent was given (2 infants), no researcher available (6 infants), and born to limited resuscitation equipment (9 infants). The 84 included infants were randomised into the 30% FiO_2 and 50% FiO_2 groups (42 infants each). Baseline maternal and infant characteristics are shown in **Table 1**. There were no significant differences between the groups.

Delivery room outcomes are shown in **Table 2**. There was a larger increase of FiO_2 supplementation during resuscitation in the 30% FiO_2 group compared to the 50% FiO_2 group. The total integrated oxygen level in the 30% FiO_2 group was significantly lower than in the 50% FiO_2 group (P=0.001).

The mean heart rates were not significantly different between the 30% and 50% FiO_2 groups, starting from the first minute to the 20th minute after birth. There were 2 infants in the low-oxygen group and 3 infants in the moderate-oxygen group who had heart rates <60 BPM after receiving 30-second positive pressure ventilation. Oxygen was increased up to 100%

Table 1. Baseline maternal and infant characteristics

Characteristics	30% FiO_2 (n=42)	50% FiO_2 (n=42)
Maternal		
Hypertension, n (%)	11 (26.2)	7 (16.7)
Diabetes mellitus, n (%)	2 (4.8)	2 (4.8)
Chronic renal failure, n (%)	0 (0.0)	0 (0.0)
Chronic heart disease, n (%)	0 (0.0)	0 (0.0)
Antenatal steroids, n (%)	24 (57.1)	30 (71.4)
Membrane rupture > 18 hours, n (%)	9 (21.4)	14 (33.3)
Clinical suspicion of chorioamnionitis, n (%)	2 (4.8)	3 (7.1)
Multiple pregnancy, n (%)	14 (33.3)	12 (28.6)
Infants		
Median gestational age (IQR), weeks	30.5 (29-32)	31(29-32)
Median birth weight (IQR), g	1,385 (1,037.5-1,632.5)	1,447.5 (1,075-1,632.5)
Sex, n (%)		
Male	13 (31.0)	20 (47.6)
Female	29 (69.0)	22 (52.4)
Method of delivery, n (%)		
Caesarian section	35 (83.3)	37 (88.1)
Vaginal	7 (16.7)	5 (11.9)

Table 2. Baseline resuscitation parameters

Parameters	30% FiO_2 (n = 42)	50% FiO_2 (n = 42)	P value
Median Apgar score (IQR)			
1st minute	6 (5-7)	6 (5-6)	0.942[c]
5th minute	8 (7-8)	8 (7-9)	0.559[c]
Heart rate <60 BPM after 90-second resuscitation, n (%)	1 (2.4)	1 (2.4)	1.000[b]
Median time to reach 88% saturation (IQR), min	4 (3-7.5)	3 (2-4.25)	0.085[c]
Increased FiO_2 during resuscitation, n (%)	12 (28.6)	4 (9.5)	0.049[a]
Median total integrated oxygen administered during resuscitation [$\sum$ (FiO_2 – 0.21) x times] (IQR), minute	41 (18-183)	115 (86-176)	0.001[c]
CPAP, n (%)	38 (91.5)	39 (92.9)	1.000[b]
PPV, n (%)	20 (47.6)	18 (42.9)	0.827[a]
ETT ventilation, n (%)	9 (21.4)	7 (16.7)	0.782[a]
Chest compression, n (%)	1 (2.4)	1 (2.4)	1.000[b]
Surfactant treatment, n (%)	0 (0.0)	5 (11.9)	0.055[a]

Note: Categorical data are presented in n (%); skewed numerical data are presented in median (interquartile range/IQR), a Chi-square test; b Fisher's test; c Mann-Whitney test, ETT = endotracheal tube

for those infants. Sixteen infants were intubated and responded satisfactorily.

The median FiO_2 concentrations during resuscitation are shown in **Figure 1**. In the low-oxygen group (30% FiO_2), the FiO_2 reached a median of 0.21 at 5 minutes after birth; while in the moderate-oxygen group (50% FiO_2), the FiO_2 reached 0.21 at 6 minutes after birth. There was a significant difference of FiO_2 administration from the first minute to the 5th minute between groups (**Figure 1**). In the low-oxygen group, 12 infants required oxygen over 30% FiO_2 to reach the target saturation and desired heart rate. In the

moderate-oxygen group, 4 infants required increased oxygen more than 50% FiO_2 (**Table 2**).

The comparison of FiO_2 between the groups are depicted in **Figure 1**. Data are presented in median because the distribution was not normal. The P values of Mann-Whitney test for each measurement were: < 0.001 (1'), < 0.001 (2'); < 0.001 (3'); < 0.001 (4'); 0.023 (5'); 0.300 (6'); 0.856 (7'); 0.364 (8'); 0.286 (9'); 0.144 (10'); 0.078 (15'); and 0.334 (20').

The SpO2 comparison between groups is shown in **Figure 2**. At the 1st to 4th minute and the 11th to the 20th minute, there was no significant difference of SpO_2 between groups. However, at the 5th to 10th minute, the moderate-oxygen group (50% FiO_2) had significantly higher saturation (SpO_2). The time needed to reach 88% SpO_2 was shorter in the 50% FiO_2 group (at the 3rd minute) than in the 30% FiO_2 group (4th minute), however, these results were not significantly different (**Table 2**).

Data are presented in median since the distribution was not normal (**Figure 2**). The number of subjects (30% FiO_2/50% FiO_2) and Mann-Whitney test P values for each measurement were: 26/23 (P=0.920) at 1'; 34/34 (P=0.277) at 2'; 39/38 (P=0.165) at 3'; 40/38 (P=0.090) at 4'; 40/41 (P=0.007) at minute 5; and 41/41 (P=0.018) at 6'. For the 7th to 20th minutes, the number of subjects was 42/42, with the following P values: P=0.033 (7'); P=0.005 (8'); P=0.052 (9'); P=0.034 (10'); P=0.431 (15'); and P=0.365 (20').

We found no significant differences in clinical outcome parameters (**Table 3**) neither in comparison of GSH/GSSG ratio and MDA between the 30% and 50% FiO2 groups (**Table 4**).

In our study, stool specimens were obtained twice, the first between days one and three of age and the second on day seven. There was no significant difference in AAT levels between the FiO_2 groups, at either time point (**Table 5**). Specimens were

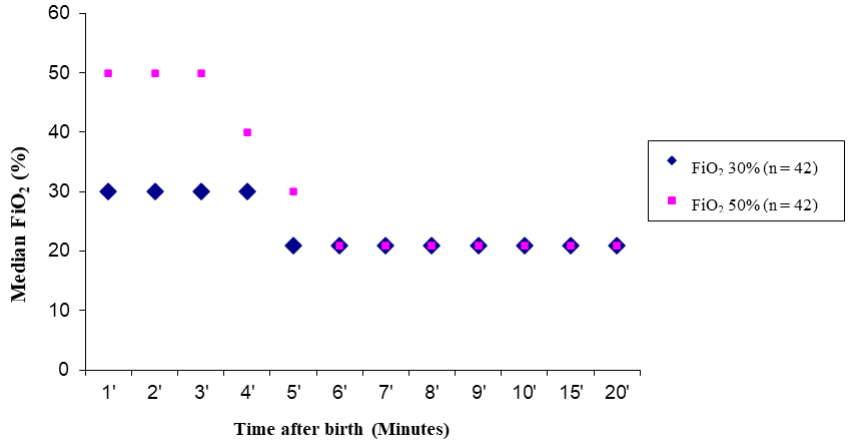

Figure 1. The comparison of FiO_2 between the groups (n = 42/group)

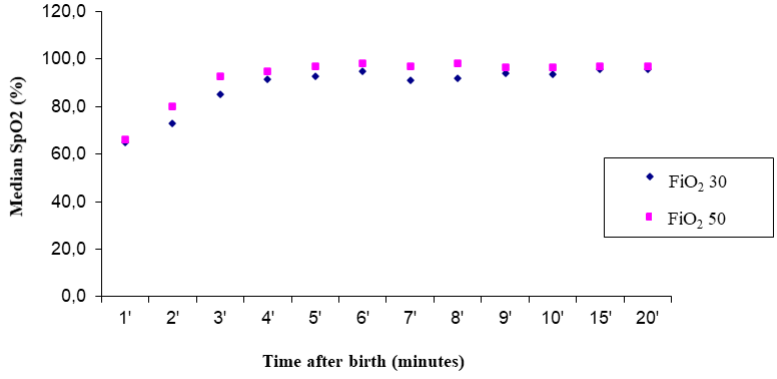

Figure 2. Comparison of SpO2 between groups for complete data per measurement

Table 3. Clinical outcome parameters

Parameters	30% FiO_2 (n=42)	50% FiO_2 (n=42)	P value
BPD (intention-to-treat analysis), n (%)	18 (42.8)	17 (40.5)	0.825[b]
BPD (per prostocol analysis) (n=32 *vs.* 31) , n (%)	8 (25)	6 (19.4)	0.285[b]
Mortality, n (%)	15 (35.7)	13 (31)	0.643[b]
Mortality at < 28 days of age, n (%)	9 (21.4)	10 (23.8)	0.794[b]
Survived without BPD, n (%)	23 (54.8)	26 (61.9)	0s.507[b]
Duration of oxygen treatment (IQR), days	11.5 (3-30.5)	9.5 (2-20.5)	0.545[a]
Duration of mechanical ventilation use (IQR), days	0 (0-5.75)	0 (0-7.75)	0.960[a]
Duration of CPAP use (IQR), days	3.5 (1-21.25)	5 (1-10.25)	0.646[a]
PDA, n (%)	17 (40.5)	10 (23.8)	0.102[b]
Pneumonia, n (%)	14 (33.3)	15 (35.7)	0.818[b]
Surfactant treatment, n (%)	8 (19.0)	5 (11.9)	0.365[b]
ROP (Grade III-IV), n (%)	1 (3.8)	1 (3.8)	1.000[c]
IVH (Grade III-IV), n (%)	4 (10.0)	2 (5.1)	0.675[c]
Sepsis, n (%)			
Proven sepsis	12 (28.6)	14 (33.3)	0.881[b]
Clinical sepsis	23 (54.8)	22 (52.4)	0.881[b]
NEC (Grade II-IV), n (%)	3 (7.14)	2 (4.76)	0.139[c]
HMD, n (%)	38 (90.5)	39 (92.9)	1.000[b]

Note: The number of subjects for ROP outcome was 26 subjects (FiO2 30%) and 26 subjects (FiO2 50%); the number of subjects for IVH outcome was 40 subjects (FiO2 30%) and 39 subjects (FiO2 50%); categorical data are presented in n (%); numerical data with skewed distribution are presented in median (quartile 1–3); a Mann-Whitney Test; bChi-square test; cFisher's test; PDA = patent ductus arteriosus; ROP = retinopathy of prematurity; IVH = intraventricular hemorrhage; NEC = necrotizing enterocolitis; HMD = hyaline membrane disease (respiratory distress syndrome)

Table 4. Comparison of GSH/GSSG ratio and MDA between the treatment groups

Parameters	FiO_2 30%	FiO_2 50%	P value
GSH/GSSG I (n = 42/42)	31.46 (30.11-32.12)	31.36 (29.92-31.79)	0.295
GSH/GSSG II (n = 41/41)	31.41 (30.09-32.10)	31.41 (30.15-32.16)	0.853
Change in GSH/GSSG	-0.05 (-3.65-24.40)	0.07 (-27.18-32.65)	0.155
MDA I (n=42/42)	0.36 (0.22-0.48)	0.32 (0.22-0.47)	0.585
MDA II (n=41/41)	0.49 (0.32-0.86)	0.52 (0.34-0.80)	0.956
Change in MDA	0.10 (-1.31-0.68)	0.15 (-0.57-2.90)	0.856

Note: Numerical data with skewed distribution are presented in median (quartile 1-3); Mann-Whitney test; GSH/GSSG I=umbilical GSH/GSSG ratio; GSH/GSSG II=GSH/GSSG ratio on the 3rd day; MDA I=umbilical MDA level; MDA II=MDA level on the 3rd day

evaluated for anaerobic bacteria (Lactobacillus and Bifidobacterium) and facultative anaerobic bacteria (*Klebsiella pneumoniae* and Acinetobacter). There was an increase in the absolute number of facultative anaerobic bacteria (*Klebsiella pneumoniae*) and anaerobic bacteria in the stool on the seventh day compared to the stool on the first day in both groups. Overall, there was no significant difference in the absolute number of anaerobic and facultative anaerobic bacteria between treatment groups (**Table 6**).

Discussion

Premature infants often require positive pressure ventilation and oxygen therapy in the delivery room.[26] Excessive oxygen exposure increases the production

Table 5. AAT level (mg/dL) of the 30% and 50% FiO_2 groups at days 1-3 and day 7

AAT level, mg/dL	30% FiO_2	50% FiO_2	P value
AAT I (n=35/37)	377.43 (74.41)	337.68 (96.80)	0.138
AAT II (n=33/38)	362.87 (82.64)	358.09 (71.92)	0.515
Change in AAT	3.00 (-321.6-103.10)	13.35 (-198.8-254.30)	0.687

Note: Numerical data with skewed distribution are presented in median (quartile 1-3); Mann-Whitney test; AAT I=AAT at day 1-3; AAT II=AAT at day 7

Table 6. Absolute number of bacteria in 200 mg stool

Microorganisms	30% FiO_2	50% FiO_2	P value
Facultative anaerobic bacteria			
Klebsiella pneumoniae I (n=40/41)	14.05 (0.00-27.45)	14.10 (0.00-32.55)	0.95
Klebsiella pneumoniae II (n=36/38)	25.65 x 102 (0.28 x 102-24.32 x 105)	20.48 x 103 (77.15-15.67 x 105)	0.53
Acinetobacter sp I (n=40/41)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.98
Acinetobacter sp II (n=36/40)	0.00 (0.00-0.98)	0.83 (0.00-9.58)	0.13
Anaerobic bacteria			
Lactobacillus sp I (n=40/41)	0.39 (0.00-1.89)	0.31 (0.00-2.02)	0.87
Lactobacillus sp II (n=36/38)	1.12 (0.29-3.57)	1.75 (0.57-9.06)	0.22
Bifidobacterium sp I (n=40/41)	24.55 (9.46-69.47)	22.60 (10.90-49.65)	0.66
Bifidobacterium sp II (n=36/38)	84.7 (24.22-392.75)	122.50 (37.50-382.00)	0.57

Note: Numerical data with skewed distribution are presented in median (interquartile range); Mann-Whitney test; I=stool on day 1-3; II=stool on day 7

of reactive oxygen species and proinflammatory cytokines.[27] We hypothesized that inflammation following the oxygen administration may damage the intestine and lead to increased AAT in the intestinal lumen. In addition, oxygen administration in the resuscitation room may increase oxygenation in the intestinal lumen and affect the intestinal microbiota.

The choice of FiO_2 of 30% was used for the control group because it is difficult to stabilize very premature infants using air; the treatment FiO_2 of 50% was based on studies which showed that resuscitation using an $FiO_2 \geq 0.8$ was associated with harmful effects.[5,6,14,28] Moreover, previous studies showed that starting resuscitation using minimal O_2 often led to the use of much higher FiO_2 (between 35% and 55%) during resuscitation. In recent years, several studies have compared different initial FiO_2 administration levels to obtain the optimum oxygen levels in premature neonatal resuscitation.[5,6,13,28-31]

Previous studies have shown similar findings to ours, with initial FiO_2 affecting the FiO_2 required in the first minutes of a resuscitation. High initial oxygen concentrations (90-100%)[5,6] and moderate oxygen (50-65%)[31] were associated with higher subsequent delivery of oxygen compared with low oxygen (21-30%), especially in the first 5 minutes of resuscitation.[5,6,31] In our study, the total amount of oxygen given during resuscitation was significantly lower in the low FiO_2 group compared to that of the moderate FiO_2 group. The moderate FiO_2 group had a shorter time to reach the 88% SpO_2 target. Our result was consistent with two previous studies which compared FiO_2 1.0 *vs.* FiO_2 0.21,[6] and 30% *vs.* 50%.[31]

With regards to oxidative stress markers, a previous study found a statistically significant difference between FiO_2 groups,[6] while another study [31] and ours found no significant differences. Free radicals cause pulmonary damage in neonates. Oxidative stress develops when the production of free radicals exceeds the antioxidant defense capacity. Oxidative stress occurs in all infants during the transition from the relatively hypoxic intrauterine to the extrauterine environment.[7] Premature infants have low antioxidant levels and, therefore, are susceptible to diseases associated with free radicals, including BPD.[9] Some studies have shown an association between the initial FiO_2 and BPD.[5,6,13,31] However, the incidence of BPD was not significantly different between the 30% and

50% FiO2 treatment groups in our study [42.8% *vs.* 40.5%, respectively (P=0.83) (intention to treat analysis) and 25% *vs.* 19.4%, respectively (P=0.285) (per protocol analysis)]. These results were similar to those of Rook *et al.*,[31] who compared an initial FiO_2 level of 30% *vs.* 65% and reported BPD incidences of 24% *vs.* 17%, respectively.

With regards to GSH/GSSG ratio, our results were also consistent those of Rook et al.,[31] with no significant difference in GSH/GSSG ratio between treatment groups. This finding may explain why there was no significant difference of BPD incidence.[32] In contrast, a study found that GSSG/GSH ratio and BPD incidence were significantly different between the 30% *vs.* 90% FiO_2 groups [15.4% *vs.* 31.7%, respectively (P<0.05)].[5] Moreover, another study showed that the level of oxidative stress and BPD incidence were significantly different between 0.21 *vs.* 1.0 FiO_2 groups (7% *vs.* 25%, respectively (P<0.05).[6] In our study, the difference in FiO_2 between the two groups was smaller and the study population was more mature compared to the the other two studies, which may explain the contrary findings.

Oxidative stress can cause inflammation of the intestinal wall, resulting in an impaired intestinal barrier.[9,10] Impaired intestinal integrity leads to loss of protein into the gastrointestinal tract.[33,34] Fecal AAT levels increase if the gut is inflamed, thus, AAT is known to be a sensitive marker of protein loss, since it is not actively secreted or absorbed by the gut, nor is it degraded.[15,16,33]

If inflammation occurs in the intestines due to high oxygen level, we would assume that the AAT level would be higher in the group receiving higher oxygen concentrations. Evaluation of AAT in fecal specimens by ELISA method is a screening test to detect abnormalities of the intestinal mucosa.[35] Our study is the first to compare AAT in the gastrointestinal tract of premature infants who had been resuscitated with moderate initial FiO_2 level compared to those who received low initial FiO_2 level. We found no significant difference in AAT levels between treatment groups either at birth or at seven days of age.

We also examined intestinal microbiota changes in the stool due to different initial oxygen delivery during resuscitation. The PCR analysis was used because it provides faster and more accurate results.[36] Anaerobic organisms are difficult to culture because they require anaerobic conditions to live and grow and a long period of time.[37,38] Anaerobic microorganisms (Bifidobacterium) were more prevalent than the facultative anaerobic microorganisms (*Klebsiella pneumoniae*) in the day three stool specimens. However, the reverse was true in stools tested at seven days. Similarly, a previous study found that anaerobic microorganisms and Streptococcus (Gram-positive bacteria) were dominant in meconium, while *Klebsiella pneumoniae, Escherichia coli*, Enterobacter, and Yersinia were more prevalent in third-day stool of premature infants.[39,40] The change from anaerobic microorganisms present at birth to anaerobic facultative microorganisms on the 7th day of life may be caused by antibiotic administration during treatment in the intensive care unit.[39,40]

In term infants, a wide range of microorganisms colonize the gastrointestinal tract in the first 10 days of life. Colonization may be delayed in premature infants because of early antibiotic administration.[41-43] In term infants, common microorganisms include Bifidobacterium, Lactobacillus, and Streptococcus,[44] while in premature infants, microorganisms include *Klebsiella pneumoniae*, Enterobacter, and *Clostridium difficile*.[45] In our study, *Klebsiella pneumoniae* was more common than Bifidobacterium, Lactobacillus, and Acinetobacter. The abundance of *Klebsiella pneumoniae* in the 7th day stool may have been due to low sensitivity to antibiotics (7.4% ampicillin-sulbactam, 11.1% gentamicin, 41.7% amikacin, and 83.9% meropenem).[46] Although there was no significant difference in anaerobic microorganisms between groups, there were more anaerobic microorganisms in the 50% FiO_2 group than in the 30% FiO_2 group in the 7th day stool. This finding may have been due to greater exposure to breast milk in the higher FiO_2 group.

Excessive oxygen exposure reduces the number of anaerobic microbes since they cannot survive in the oxygen-rich environment, potentially leading to increased number of facultative anaerobic microorganisms and, subsequently, inducing an intestinal microbiota imbalance. Using a pig model, Albenberg *et al.*[47] studied the effect of oxygen level on the composition of fecal microbiota and microorganisms in the intestinal mucosa. They showed that after oxygen supplementation, there

was a consistent increase in facultative anaerobic microorganism colonization.

We noted no significant difference in the absolute number of anaerobic and facultative anaerobic bacteria between the 30% and 50% FiO_2 groups. This finding was to be expected, because 50% FiO_2 was used for a short time (only during resuscitation), while a previous study used hyperbaric oxygen (100% oxygen at 2.0 atm pressure) for 2 hours each day for 9 days.[47] The results showed an increase in the number of oxygen-tolerant organisms. To our knowledge, there have been no studies in infants to date comparing the microbiota before and after administration of oxygen.

A limitation of our study was that caregivers were unblinded, hence, it is possible that the two groups received different resuscitative measures. However, heart rate, FiO_2, and SpO_2 data were derived objectively from video recordings. In addition, our sample size was small, so important differences in primary and secondary outcomes may have gone undetected.

Most previous studies comparing FiO_2 delivery were conducted in developed countries. Our study was conducted in a developing country, so the results may be applicable to other developing countries where rates of antenatal steroid use are similarly modest (65% in our case). But our chief strength was that subjects were assigned by random allocation. Moreover, this study is the first to investigate the intestinal barrier using AAT as a biological marker and report the intestinal microbiota in premature infants resuscitated with different initial oxygen concentration. Although AAT examination and intestinal microbiota were secondary data, we found that AAT was high in premature infants at birth.

In conclusion, in very premature infants between 25-32 weeks of gestational age resuscitated using an initial FiO_2 of 30% or 50%, we find no significant differences in rates of BPD, oxidative stress markers (GSH/GSSG ratio and MDA levels), intestinal mucosal integrity (AAT levels), and intestinal microbiota. This trial provides baseline data to allow for more accurate sample size calculations for future trials. Further well-designed studies of adequate size and power are needed.

Conflict of interest

None declared.

Funding acknowledgment

The authors received no specific grants from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Kattwinkel J, American Heart Association, American Academy of Pediatrics. Kattwinkel K, McGowan JE, Zaichkin J, editors. Textbook of neonatal resuscitation. Elk Grove Village: AAP; 2011. ISBN: 9781581104981. p.2.
2. Northway WH Jr, Rosan RC, Porter DY. Pulmonary disease following respirator therapy of hyaline-membrane disease. Bronchopulmonary dysplasia. N Engl J Med. 1967;276:357-8. DOI: 10.1056/NEJM196702162760701.
3. Campbell K. Retrolental fibroplasia. Med J Aust. 1971;2:282.
4. Tin W, Gupta S. Optimum oxygen therapy in preterm babies. Arch Dis Child Fetal Neonatal Ed. 2007;92:F143-7. DOI: 10.1136/adc.2005.092726.
5. Vento M, Moro M, Escrig R, Arruza L, Villar G, Izquierdo I, *et al.* Preterm resuscitation with low oxygen causes less oxidative stress, inflammation, and chronic lung disease. Pediatrics. 2009;124:e439-49. DOI:10.1542/peds.2009-0434.
6. Kapadia VS, Chalak LF, Sparks JE, Allen JR, Savani RC, Wyckoff MH. Resuscitation of preterm neonates with limited versus high oxygen strategy. Pediatrics. 2013;132:e1488-96. DOI:10.1542/peds.2013-0978.
7. Forman HJ, Fukuto JM, Miller T, Zhang H, Rinna A, Levy S. The chemistry of cell signaling by reactive oxygen species and nitrogen species and 4-hydroxynonenal. Arch Biochem Biophys. 2008;477:183-95. DOI: 10.1016/j.abb.2008.06.011.
8. Escobar J, Cernada M, Vento M. Oxygen and oxidative stress in the neonatal period. Neoreviews. 2011;12:e613-24. DOI:10.1542/neo.12-11-e613.
9. Perrone S, Tataranno ML, Negro S, Longini M, Marzocchi B, Proietti F, *et al.* Early identification of the risk for free radical-related diseases in preterm newborns. Early Hum Dev. 2010;86:241-4. DOI: 10.1016/j.earlhumdev.2010.03.008.
10. Friel JK, Friesen RW, Harding SV, Roberts LJ. Evidence of oxidative stress in full-term healthy infants. Pediatr Res. 2004;56:878-82. DOI: 10.1203/01.PDR.0000146032.98120.43.

11. Pitkanen OM, O'Brodovich HM. Significance of ion transport during lung development and in respiratory disease of the newborn. Ann Med. 1998;30:134-42. DOI: 10.3109/07853899808999396.
12. MacNee W. Oxidants/antioxidants and COPD. Chest. 2000;117:303-17. doi: 10.1378/chest.117.5_suppl_1.303s-a.
13. Wang CL, Anderson C, Leone TA, Rich W, Govindaswami B, Finer NN. Resuscitation of preterm neonates by using room air or 100% oxygen. Pediatrics. 2008;121:1083-9. DOI: 10.1542/peds.2007-1460.
14. Escrig R, Arruza L, Izquierdo I, Villar G, Saenz P, Gimeno A, *et al.* Achievement of targeted saturation values in extremely low gestational age neonates resuscitated with low or high oxygen concentration: a prospective, randomized trial. Pediatrics. 2008;121:875-81. DOI: 10.1542/peds.2007-1984.
15. Braamskamp MJ, Dolman KM, Tabbers MM. Clinical practice. Protein-losing enteropathy in children. Eur J Pediatr. 2010;169:1179-85. DOI: 10.1007/s00431-010-1235-2.
16. Lisowska-Myjak B. AAT as a diagnostic tool. Clin Chim Acta. 2005;352:1-13. DOI: 10.1016/j.cccn.2004.03.012.
17. Carrel RW. Alpha 1-antitrypsin: molecular pathology, leukocytes, and tissue damage. J Clin Invest. 1986;78:1427-31. DOI: 10.1172/JCI112731.
18. Mitsuoka T, Kaneuchi C. Ecology of the bifidobacteria. Am J Clin Nutr. 1977;30:1799-810. DOI: 10.1093/ajcn/30.11.1799.
19. Balmer SE, Wharton BA. Diet and faecal flora in the newborn: breast milk and infant formula. Arch Dis Child. 1989;64:1672-77. DOI: 10.1136/adc.64.12.1672.
20. Berg RD. Bacterial translocation from the gastrointestinal tract. Trends Microbiol. 1995;3:149-54. DOI: 10.1016/s0966-842x(00)88906-4.
21. Ehrenkranz RA, Walsh MC, Vohr BR, Jobe AH, Wright LL, Fanaroff AA, *et al.* Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics. 2005;116:1353-60. DOI:10.1542/peds.2005-0249.
22. O'Donnell CP, Kamlin CO, Davis PG, Morley CJ. Obtaining pulse oximetry data in neonates: a randomised crossover study of sensor application techniques. Arch Dis Child Fetal Neonatal Ed. 2005;90:84-5. DOI: 10.1136/adc.2004.058925.
23. Perlman JM, Wyllie J, Kattwinkel J, Atkins DL, Chameides L, Goldsmith JP, *et al.* Part 11: neonatal resuscitation: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Circulation. 2010;122:S516-38. D OI: 10.1161/CIRCULATIONAHA.110.971127.
24. Repetto M, Semprine J, Boveris, A. Lipid peroxidation: chemical mechanism, biological implications and analytical determination. In: Catala, A. , editor. Lipid peroxidation [Internet]. London: IntechOpen; 2012 [cited 2019 November]. Available from: https://www.intechopen.com/chapters/38477. DOI: 10.5772/45943.
25. Oxford Biomedical Research. Microplate assay for GSH/GSSG (reduced/oxidized glutathione) [Internet]. 2008 [cited 2019 November 13]. Available from: https://www.oxfordbiomed.com/sites/default/files/spec_sheet/GT40.pdf. p. 1-7.
26. Leone TA, Rich W, Finer NN. A survey of delivery room resuscitation practices in the United States. Pediatrics. 2006;117:164-75. DOI: 10.1542/peds.2005-0936
27. Jobe AH, Hillman N, Polglase G, Kramer BW, Kallapur SH, Pillow J. Injury and inflammation from resuscitation of the preterm infant. Neonatology. 2008;94:190-6. DOI: 10.1159/000143721
28. Lundstrom KE, Pryds O, Greisen G. Oxygen at birth and prolonged cerebral vasoconstriction in preterm infants. Arch Dis Child Fetal Neonatal Ed. 1995;73:F81-6. DOI: 10.1136/fn.73.2.f81
29. Rook D, Schierbeek H, van der Eijk AC, Longini M, Buonocore G, Vento M, *et al.* Resuscitation of very preterm infants with 30% vs. 65% oxygen at birth: study protocol for a randomized controlled trial. Trials. 2012;13:65. DOI: 10.1186/1745-6215-13-65
30. Rabi, Y, Singhal N, Nettel-Aguirre A. Room-air versus oxygen administration for resuscitation of preterm infants: the ROAR study. Pediatrics. 2011;128:e374-81. DOI: 10.1542/peds.2010-3130.
31. Rook D, Schierbeek H, Vento M, Vlaardingerbroek H, Eijk AC, Longini M, *et al.* Less stress: oxidative stress and gluthatione kinetics in preterm infants. Rotterdam: Erasmus University Rotterdam; 2013. p. 62-73.
32. Buonocore G, Perrone S. Biomarkers of oxidative stress in the fetus and newborn. Haematol Rep. 2006;2:103-7.
33. Tangsilsat D, Atamasirikul K, Treepongkaruna S, Bed SN, Sumritsopak R, Kunakorn M. Fecal alpha1-antitrypsin in healthy and intestinal-disorder Thai children. J Med Assoc Thai. 2007;90:1317-22.
34. Oswari H, Prayitno L, Dwipoerwantoro PG, Firmansyah A, Makrides M, Lawley B, *et al.* Comparison of stool microbiota compositions, stool alpha1-antitrypsin and calprotectin concentrations, and diarrhoeal morbidity of Indonesian infants fed breast milk or probiotic/prebiotic-supplemented formula. J Paediatr Child Health. 2013;49:1032-9. DOI: 10.1111/jpc.12307.
35. Darani HY, Rahimian G, Nafisi M, Amini SA, Najafi A, Sarafpoor M. Unsuitability of fecal alpha 1-antitrypsin as a

marker for differentiation of microbial and non-microbial diarrhea. Kuwait Med J. 2005; 37:91-3.
36. Penders J, Thijs C, Vink C, Stelma FF, Snijders B, Kummeling I, et al. Factors influencing the composition of the intestinal microbiota in early infancy. Pediatrics. 2006;118:511-21. DOI: 10.1542/peds.2005-2824.
37. Favier CF, Vaughan EE, De Vos WM, Akkermans AD. Molecular monitoring of succession of bacterial communities in human neonates. Appl Environ Microbiol. 2002;68:219-26. DOI: 10.1128/AEM.68.1.219-226.2002.
38. Millar MR, Linton CJ, Cade A, Glancy D, Hall M, Jalal H. Application of 16S rRNA gene PCR to study bowel flora of preterm infants with and without necrotizing enterocolitis. J Clin Microbiol. 1996;34:2506-10. DOI: 10.1128/jcm.34.10.2506-2510.1996.
39. Harris MC, D'Angio CT, Gallagher PR, Kaufman D, Evans J, Kilpatrick L. Cytokine elaboration in critically ill infants with bacterial sepsis, necrotizing entercolitis, or sepsis syndrome: correlation with clinical parameters of inflammation and mortality. J Pediatr. 2005;147:462-8. DOI: 10.1016/j.jpeds.2005.04.037
40. Harding D. Impact of common genetic variation on neonatal disease and outcome. Arch Dis Child Fetal Neonatal Ed. 2007;92:408-13. DOI: 10.1136/adc.2006.108670.
41. Gewolb IH, Schwalbe RS, Taciak VL, Harrison TS, Panigrahi P. Stool microflora in extremely low birthweight infants. Arch Dis Child Fetal Neonatal Ed. 1999;80:167-73. DOI: 10.1136/fn.80.3.f167
42. Rook GA, Brunet LR. Microbes, immunoregulation, and the gut. Gut. 2005;54:317-20. DOI: 10.1136/gut.2004.053785.
43. Huurre A, Kalliomäki M, Rautava S, Rinne M, Salminen S, Isolauri E. Mode of delivery-effects on gut microbiota and humoral immunity. Neonatology. 2008;93:236-40. DOI: 10.1159/000111102.
44. Matamoros S, Gras-Leguen C, Le Vacon F, Potel G, de La Cochetiere M-F. Development of intestinal microbiota in infants and its impact on health. Trends Microbiol. 2013;21:167-73. DOI: 10.1016/j.tim.2012.12.001.
45. Arboleya S, Binetti A, Salazar N, Fernandez N, Solis G, Hernandez-Barranco A, *et al.* Establishment and development of intestinal microbiota in preterm neonates. FEMS Microbiol Ecol. 2012;79:763-72. DOI: 10.1111/j.1574-6941.2011.01261.x
46. Division of Infectious Diseases, Department of Clinical Pathology Cipto Mangunkusumo General Hospital. Bacterial and antibiotics susceptibility profile at cipto mangunkusumo general hospital. Jakarta: Rumah Sakit Cipto Mangunkusumo (RSCM); 2015. p. 98-100.
47. Albenberg L, Esipova TV, Judge CP, Bittinger K, Chen J, Laughlin A, *et al.* Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. Gastroenterology. 2014;147:1055-63. DOI: 10.1053/j.gastro.2014.07.020.