PRETERM ve TERM İNFANTLARDA İNHALE NİTRİK OKSİT SONUÇLARI: ÜÇÜNCÜ DÜZEY YENİDOĞAN YOĞUN BAKIM ÜNİTESİNDEKİ DÖRT YILLIK DENEYİM

Outcomes of Inhaled Nitric Oxide in Preterm and Term Infants: A Four-Year Experience in a Tertiary Neonatal Unit

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ÖZ

GİRİŞ ve AMAÇ: Bu çalışmada pulmoner hipertansiyon (PH) kliniği ile karakterize olan preterm ve term infantların, inhale nitrik oksit (iNO) tedavisine yanıtlarındaki etkinliğin değerlendirilmesi amaçlanmıştır.

YÖNTEM ve GEREÇLER: Çalışma kohortu 2015-2019 yılları arasında iNO tedavisi alan hastalardan oluşmuştur, hastalar gestasyon haftaları ve PH tanısının zamanına göre sınıflandırılmıştır.

BULGULAR: Çalışmaya toplamda 56 vaka dahil edilmiştir. iNO öncesi ve iNO sonrası ortalama hava yolu basınç değerleri ve oksijenasyon indeksi preterm infantların grubunda anlamlı düzeyde yüksek bulundu ($p < 0.05$). Erken PH tanısı alan grupta ortalama gestasyon haftası (GH) ve doğum ağırlığı (DA) daha yüksek bulundu. İNO tedavisine yanıt veren grupta GH'ları ve DA'nın belirgin yüksek oldugu izlendi ($p < 0.05$). İNO tedavisine yanıt alınamayan hastaların genellikle tanıları term bebeklerde diafragma hernisi iken pretermlerde sepsisdi.

TARTIŞMA ve SONUÇ: Term infantlarda İNO tedavisine daha iyi yanıt alınmakla beraber pretermlerde de İNO tedavisine erken başlanması ile daha iyi yanıt alınabilir.

Anahtar Kelimeler: inhale nitrik oksit, preterm, pulmoner hipertansiyon

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ABSTRACT

INTRODUCTION: This study aimed at assessing the data of term and preterm neonates with pulmonary hypertension (PH) to characterise patients and the effectiveness of inhaled nitric oxide (iNO).

MATERIALS and METHODS: The study cohort treated with iNO between 2015 and 2019 was categorized into two groups according to the gestational age (GA) and timing of PH.

RESULTS: Overall 56 neonates were enrolled. Pre-iNO, post-iNO mean airway pressure values, and oxygenation index were significantly higher in the preterm group ($p < 0.05$). Mean GA and birth weight of the infants who had early PH diagnosis were higher. Positive responders were more likely to have higher GA as well as higher birth weight ($p < 0.05$). Non-responders were mostly diagnosed with diaphragmatic hernia in term infants and with sepsis in preterm infants

CONCLUSIONS: Despite term infants have higher positive response rate to treatment, initiating early treatment will increase the rate of positive response in preterm infants.

Key words: inhale nitric oxide, preterm, pulmonary hypertension

INTRODUCTION

Nitric oxide (NO) molecule was found out 30 years ago and shortly after the discovery of NO, known as endothelial-derived relaxing factor, studies showed that it is a pulmonary-specific vasodilator (1). NO administered via inhalation (iNO) is a fat soluble, unstable, free radical gas. It is a small molecule that can be easily transferred through cell membranes; its half life is short and is expressed in seconds (2). Endogenous NO synthesis in neonates is induced by various events such as birth, stress, oxygen increase, or sepsis and formed via nitric oxide synthase, and it causes vasodilatation in smooth muscle cells via cGMP. iNO provides selective vasodilation in the pulmonary veins with its selective effect characteristic, and it also increases blood flow in the alveoli by decreasing the ventilation perfusion mismatch with its micro-selective effect (3).

The use of iNO improves oxygenation in neonates with persistent pulmonary hypertension (PH) (4). Persistent PH arises in neonates either as a primary or secondary condition to respiratory distress syndrome, meconium aspiration, sepsis, or birth asphyxia. (5-7). It is characterized by persistently increased pulmonary vascular resistance and arterial pressure that is associated with extrapulmonary right-to-left shunting leading to hypoxemic respiratory failure in tertiary neonatal intensive care units (NICUs). iNO is an effective but costly therapy which was approved for only late preterm and term infants with PH; however, off-label use of iNO has become widespread as 5%–7% cases in preterm infants. (8, 9). In term and preterm infants, iNO is used to treat PH caused by various reasons (10). Cochrane review reported that iNO use improves oxygenation in term and late preterm infants, whereas it does not have an effect on mortality and length of hospital stay and is also ineffective in smaller preterm infants (11, 12).

The primary objective of this study is to characterize the term and preterm cases in whom we used iNO for 4 years in the neonatal intensive care unit. The secondary objective is to identify factors associated with improved outcomes and favourable response to iNO therapy and to evaluate our iNO practice.

MATERIALS and METHODS

A retrospective-cohort study was conducted at the University of Health Sciences, Bursa Yuksek Ihtisas Teaching Hospital between 2015 and 2019 after approval of the study by the local ethics committee. We included term and preterm infants treated with iNO in our NICU. Infants who had major congenital cardiac as well as other major anomalies were excluded. The infants were categorized into two groups: Group 1, gestation age (GA) <34 weeks and Group 2, GA ≥34 weeks. iNO treatment was initiated under inadequate oxygen supply conditions with conventional mechanical ventilation strategies or severe respiratory failure [mean airway pressure (MAP) of ≥10 or oxygenation index of ≥25]. Oxygenation index (OI) was calculated as (MAP [cm H_2O] × fraction of inspired oxygen [FIO_2] × 100)/partial pressure of oxygen in arterial blood (PaO_2) (mmHg). Measured oxygen saturation difference between the pre-ductal and post-ductal points in all infants was above 10%. Echocardiography (ECHO) was performed in some patients to measure PH and make a definite diagnosis and exclude the diagnosis of congenital heart diseases. iNO treatment was continuously administered intratracheally during mechanical ventilation. The treatment was started at a dose of 20 ppm, and patients who had FiO_2 reduced to ≥0.20 during the 1st hour of treatment were considered as positive responders. In positive responders, the treatment was kept at 20 ppm until OI reached <10 and FiO_2 reached <60; thereafter, weaning was decreasing every 4 to 6 hours. After 1 hour, if FiO_2 level did not change or decreased to <0.10 despite the treatment, the treatment was terminated according to the protocol. The patients were classified into two groups according to the initiation time of iNO treatment as early (iNO initiation at ≤3 days of age) or late (at >3 days of age).

The following data were recorded: maternal medical history, prenatal demographics, antenatal steroid administration, GA, birth weight, gender, mode of delivery, Apgar score at 1st and 5th min, diagnoses, hospital stay durations, and mortality data. In addition, data related to iNO use; initiation time, treatment responses, treatment duration and MAP before (pre-iNO) and 2 hours after (post-iNO), FiO_2, and OI were recorded. Based on these values, intra- and inter-group comparisons were performed to determine the "iNO responder" group.

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Statistical analyses: Statistical analyses of the data were performed using the Statistical Package for the Social Sciences (SPSS) version 20.0 software (SPSS Inc., Chicago, IL, USA). All the continuous values were presented as the median (interquartile range, IQR) and mean ± standard deviation, where suitable. The categorical values were presented as the frequency and percentage. Chi-square analysis and Fisher's exact test was used for comparing categorical variables among the groups. Student's t-test was used for continuous variables and Mann–Whitney U was used for comparing nonparametric variables. Binary logistic regression (forward logistic regression) was used in the multivariate analysis to determine the factors associated with positive responders. Variables included in the analyses were gestational age, birth weight, sex, early PHT, PHT-definite, pre-iNO MAP, pre-iNO oxygenation index, duration of ventilation and oxygen treatment days, length of hospital stay and mortality. Statistical significance was set at $p < 0.05$.

RESULTS

Overall 56 neonates received iNO at the NICU during the 4-year study. After excluding infants meeting the exclusion criteria, 44 neonates were included in the final analysis. A total of 33 (75%) cases were classified as definite PH based on echocardiography results. Of the analysed patients, 32 (72%) were in the early PH group, whereas 12 (28%) were in the late PH group. Although mean gestational age and birth weight of the infants diagnosed with early PH were higher, no significant difference was observed. Clinical diagnoses of early and late PH groups are shown in Table 1.

Table 1: Neonatal and maternal characteristics of early and late pulmonary hypertension groups

	Early PH (n=32)	Late PH (n=12)	P
GA at birth, wk median (IQR)	36 (32-38)	32 (26-38)	0.07[a]
BW, g median (IQR)	2528±936	1857±1129	0.05[b]
Sex, n (%) Male	20 (62)	6 (50)	0.4[c]
C/S delivery, n (%)	23 (72)	6 (66)	0.8[c]

Apgar score, median (IQR)
  Minute 1
  Minute 5
	6 (5-7)
	7 (6-8)
		5 (4-6)
		6(5-7)
			0.2[a]
			0.2[a]

Antenatal steroid, n (%)	6 (19)	3(25)	0.8[c]

Maternal risk factors
  Hypertension
  Gestational diabetes
  Abruptio plasenta
  PPROM
	
	4 (13)
	3 (9)
	3 (9)
	4 (13)
		
		3 (25)
		1 (8)
		1 (8)
		4 (33)
			
			0.3[c]
			0.9[c]
			0.9[c]
			0.1[c]

Diagnosis MAS Sepsis Diaphragmatic hernia HIE RDS BPD TTN	 2 (6) 8 (25) 9 (28) 5 (16) 6 (19) 2 (6)	 2 (17) 3 (25) 2 (17) 4 (33) 1 (8)	0.1[c]

[a] Mann-Whitney U test, [b] Student t test, , [c] Chi Square test
PH: pulmonary hypertension; GA: gestational age; wk: week; IQR: interquartile range; BW: birth weight; g: gram; C/S: cesarean section; PPROM: preterm prolonged rupture of membranes; MAS: Meconium aspiration syndrome HIE: hypoxic ischemic encephalopathy, RDS: Respiratory distress syndrome, BPD: bronchopulmonary dysplasia; TTN: Transient Tachypnea of the Newborn

After the infants were classified according to birth weeks, 17 (39%) were at <34 gestation weeks (group 1) and 27 (61%) were at >34 gestation weeks (group 2). After comparing these groups, pre-iNO, post-iNO, MAP, pre-iNO, and OI values were found to be significantly higher in group 1. Age at PH was significantly different with averages being at the third day in Group 1 and first day in Group 2 (p = 0.04). As we expected, length of stay was longer in Group 1 (p = 0.03). Furthermore, iNO response was better among infants with higher gestational age (p = 0.04). Clinical and laboratory findings of the groups are presented in Table 2.

Table 2: Clinical and laboratory findings of the groups

	Grup 1 (n=18)	Grup 2 (n=26)	p
Received surfactant, n (%)	16 (89)	10 (39)	**0.001[a]**
Duration of ventilation (days)	16 (9-35)	9 (7-11)	**0.005[b]**
Duration of 02 treatment (days)	22 (9-56)	10 (8-14)	**0.01[b]**
Age at PH (days)	3 (1-15)	1 (1-2)	0.07[b]
PH- definite	13 (72)	20 (77)	0,7[a]
Duration of iNO use (hours)	84 ± 40	88 ± 42	0.7[c]
HFO at ino	7 (39)	11 (42)	0.8[a]
Pre-iNO mean airway pressure (cmH2O)	13 (13-14)	12 (11-13)	**0.01[b]**
Pre iNO FiO2	1.0 (1.0-1.0)	1.0 (1.0-1.0)	0.7[b]
Pre iNO OI	32 (29-40)	30 (26-37)	**0.04[b]**
Post-iNO mean airway pressure (cmH2O)	13 ± 1	12 ± 1	**0.03[c]**
Post-iNO FiO2	0.8 (0.7-0.9)	0.8 (0.7-0.9)	0.9[b]
Post-iNO OI	25 ± 6	22 ± 8	0.1[c]
İNO responsiveness	7 (39)	18 (69)	**0.04[a]**
Pulmonary hemorrhage	4 (22)	4 (15)	0.8[a]
≥ Grade 3 intraventricular haemorrhage	4 (22)	1 (4)	0.06[a]
Length of hospital stay (days)	24 (9-69)	15 (9-19)	**0.04[b]**
Early PH	10 (56)	22 (84)	**0.03[a]**
Mortalite	6 (33)	6 (23)	0.4[a]

[a]Chi Square test, , [b]Mann-Whitney U test, [c]Student-T test. PH: pulmonary hypertension, iNO: inhaled nitric oxide, HFO: high frequency ventilation, FiO2: fraction of inspired oxygen, OI: oxygenation index

When the infants born with gestational age before 34 weeks were compared according to the iNO initiation day, duration of ventilation days, and O_2 treatment days were significantly less (p = 0.004 and p = 0.02 respectively) in the early PH group. In early PH group, nine (90%) cases were classified as definite PH based on echocardiography results and only one (10%) from the documented pre-iNO pre–post ductal saturation difference (p = 0.02). Although there was no statistical significance, positive response rate was higher in the early PH group [5/10 (50%) vs. 2/8 (25%)]. Mortality was less in the early PH group compared to the late PH group (Table 3).

Table 3: Clinical and laboratory findings of the preterm infants

	Early PH (n=10)	Late PH (n=8)	p
Received surfactant, n (%)	9 (90)	7 (88)	0.9[a]
Duration of ventilation (days)	13 ± 10	57 ± 48	**0.04[c]**
Duration of 02 treatment (days)	23 ± 20	61 ± 53	**0.04[c]**
Age at PH (days)	1(1-3)	17 (9-50)	**0.001[c]**
PH- definite	9 (90)	3 (38)	0,02[a]
Duration of iNO use (hours)	75 ± 42	94 ± 38	0.3[c]
HFO at ino	3 (30)	4 (50)	0.4[a]
Pre-iNO mean airway pressure (cmH2O)	13 ± 2	13 ± 1	0.8[b]
Pre iNO FiO2	1 (1-1)	1 (0.9-1)	0.8[c]
Pre iNO OI	33 ± 6	32 ± 7	0.8[b]
Post-iNO mean airway pressure (cmH2O)	13 ± 1	12 ± 1	0.6[b]
Post-iNO FiO2	0.8 ± 0.1	0.8 ± 0.1	0.6[b]
Post-iNO OI	25 ± 6	24 ± 5	0.9[b]
Pulmonary hemorrhage	2 (20)	2 (25)	0.8[a]
≥ Grade 3 intraventricular haemorrhage	2 (20)	2 (25)	0.8[a]
Length of hospital stay (days)	16 (5-56)	35 (18-23)	0.1[c]
İNO responsiveness	5 (50)	2 (25)	0.3[a]
Mortalite	2 (20)	4 (50)	0.2[a]

[a]Chi Square test, , [b]Student-T test, [c]Mann-Whitney U test, PH: pulmonary hypertension, iNO: inhaled nitric oxide, HFO: high frequency ventilation, FiO2: fraction of inspired oxygen, OI: oxygenation index

When all patients were evaluated, positive response rate to iNO treatment was 57%, whereas in term infants, positive response rate was 70%. Positive responders were more likely to be with higher GA and higher birth weight (Table 4).

Table 4: Comparision of baseline characteristics of positive responders

	Positive responder (n=25)	Non-responder (n=16)	p
GA at birth, wk median (IQR)	36 (33-39)	31 (26-37)	**0.02[a]**
BW, g median (IQR)	2525 ± 790	2100 ± 1253	**0.04[b]**
Sex, n (%) Male	15 (60)	11 (58)	0.9[c]
Early PH	20 (80)	12 (63)	0,2[c]
PH-Definite	20 (80)	13 (68)	0,4[c]
Pre-ino mean airway pressure	12 (11-14)	13 (12-13)	0,3[a]
Pre-ino Oİ	28 (26-30)	34 (30-40)	**0.04[a]**
Duration of ventilation (days)	9 (7-12)	12 (8-28)	0,1[a]
Duration of 02 treatment (days)	12 (8-17)	15 (9-31)	0,4[a]
Length of hospital stay (days)	15(11-28)	19 (9-35)	0.6[a]
Mortalite	4 (16)	8 (42)	**0.04[c]**

[a]*Mann-Whitney U test,* [b]*Student-T test,* [c]*Chi Square test GA: gestational age; wk: week; IQR: interquartile range; BW: birth weight; g: gram; PH: pulmonary hypertension, iNO: inhaled nitric oxide, OI: oxygenation index;*

According to forward binary logistic regression results, only GA and birth weight were significant factors for positive responders. GA was significant risk factor with odds ratio [OR] = 1.869 [95% confidence interval (CI): 1.204–2.901; $p = 0.005$], whereas birth weight was preventive factor with OR = 0.998 (CI: 0.996–0.999; $p = 0.036$) for positive responders. When the clinical diagnoses of patients who did not respond to iNO treatment were examined, it was observed that the non-responders 'rate was the highest in the diaphragmatic hernia (4/8) group in term infants and in sepsis (4/11) group in preterm infants.

DISCUSSION

Inhaled NO therapy effectively improves oxygenation in term infants with hypoxic respiratory failure and persistent PH (4, 13). However, the indication for iNO therapy is not well defined in preterm infants. Furthermore, the characteristics and diagnoses of infants responding to iNO treatment are not clear. In this study, we aimed to identify our iNO experience in term and preterm infants admitting at our neonatal intensive care unit throughout 4 years, the characteristics of our patient groups, and the factors associated with favourable response to iNO therapy.

The neonatal pulmonary vascular system is generally unstable. The maturation of endothelial cell function, release of vasoactive mediators, vascular wall remodelling, and smooth muscle cell differentiation are involved in the maturation process (14). Neonatal PH is caused by a variety of factors including maternal anemia, fever, lung disease, diabetes mellitus, meconium- stained amniotic fluid, perinatal asphyxia, fetal distress, and sepsis (15). iNO is a selective pulmonary vasodilator and is used for treating early persistent PH in term infants. In preterm infants, it is especially used in respiratory failure, respiratory distress syndrome (RDS), or bronchopulmonary dysplasia (BPD) that does not respond to conventional treatments (16). In our study, GA and birth weight of the neonates in early PH group were higher than the late PH group. The most frequent diagnosis in the early PH cohort was diaphragmatic hernia, whereas the most frequent in the late PH cohort was BPD.

The National Institutes of Health consensus panel and the American Academy of Pediatrics Committee on Fetus and Newborn has reported that there is no sufficient evidence to recommend the routine use of iNO in preterm infants with GA <34 weeks (17, 18). However, in the guideline published by the American Heart Association and the American Thoracic Society, it was reported that iNO therapy can be beneficial especially for EMR and oligohydramnios infants with PH secondary to hypoxemia in preterm (19). In the recent years, there have been studies showing that iNO use is effective in preterm infants at least as much as in term infants (20). Due to these conflicting reports, we evaluated our own patient group. Majority of our patient group was formed by infants with GA >34 weeks. Early PH diagnosis was apparently defined more for infants with GA >34 weeks. This was most probably caused by the diagnoses of the patients in this group with hypoxic ischemic encephalopathy and diaphragmatic hernia encountered with early PH clinic. As expected, received surfactant rate,

duration of ventilation days, length of hospital stays and iNO responsiveness of the group with GA <34 weeks were higher. When the OI and MAP values of the groups before and after iNO were compared, it was observed that pre-iNO MAP and OI values were higher in the infants with GA <34 weeks. This made us consider that we may have been late in starting the iNO treatment in preterm infants which decreased our iNO treatment success in preterm infants. In a recently published study, it was shown that patients between OI 15–25 have better clinically significant improvements and mortality compared to the OI of >25 group (21). Further studies are needed with large case series comprising various OI groups to prove this hypothesis.

In preterm infants, unlike term neonates, we observed pulmonary diseases to be a secondary particularly to RDS. The prevalence of RDS causing oxygenation failure has now decreased after antenatal steroids and postnatal surfactant therapy (22). In preterm infants, other than RDS, sepsis, persistent fetal circulation, or BPD can damage the pulmonary vascular structure which may clinically encountered PH. (10). In animal studies, it was shown that NO synthase expression decreases in the epithelium of small airways as well as in the endothelium of the distal pulmonary artery in chronic pulmonary disease (23). At the same time tracheal aspirate analysis in hypoxemic PH preterm infants with PPROM or sepsis has shown a transient defect in endogenous nitric oxide generation (22). In our study, RDS along with PH secondary to sepsis in the early period and PH associated with BPD in the late period were detected in the preterm infants. In compliance with the literature, PPROM and oligohydramnios prevalence were higher in the preterm group due to the need for iNO. Due to this treatment is off-label in preterm infants with GA <34 weeks, there are very few studies concerning this area (6,10,16). In our study, early PH group with GA <34 weeks were monitored with less oxygen treatment and ventilation days and definite-PH diagnoses were more. This was caused by the late PH group consisting mostly of the BPD diagnosis and these being started with the treatment without the need for definite diagnosis with ECHO.

iNO response in PH treatment is associated with gestational age in neonates. Cochrane meta-analysis states that iNO therapy in term and near-term infants, OI decreased by a mean of 15.1 within 30–60 min after the start of therapy (12). However, Cochrane meta-analysis states that in preterm infants, early routine use of iNO did not appear to be effective but later use of iNO to prevent BPD could be effective (11). In our study, the positive response rate to iNO treatment was 57% in patients who received iNO and 70% for term infants. The evaluation of factors associated with iNO responsiveness according to forward binary logistic regression

analysis showed that only GA and birth weight were significant factors for positive responders. GA was significant risk factor, whereas birth weight was preventive factor for positive responders. Moreover, preliminary OI values and mortality rates in the iNO responder group were lower. When the clinical diagnoses of the patients who were non-responders to the iNO treatment were evaluated, it was observed as consistent with the literature that the worst responses were in the diaphragmatic hernia group in term infants and the sepsis group in the preterm infants.

There are limitations of this study where first one is its retrospective design; however, we were able to use a highly reliable neonatal database that identified all the infants with PH. As a second one, we did not have a control group of untreated infants, so we cannot confirm the impact of iNO on neonatal outcomes. In addition, we could not determine subgroups of different pre-iNO OI and MAP values which is the third limitation. As a final one, our sample size was relatively small due to iNO use which is limited in the NICU and even less in preterm infants. Despite these limitations, there are powerful strengths of our study that are an important addition to the limited clinical study data available in preterm infants with PH and provide valuable insight into the actual use of iNO.

Conclusion: In conclusion, iNO is used for treating neonatal PH in term and preterm infants. As it is an off-label treatment in preterm infants, the parameters for iNO treatment initiation decision are quite high. This can decrease iNO treatment success in neonates in critical condition who would not have the chance to be given ECMO, such as preterm infants. Thus, iNO use should be optimized in preterm infants. Therefore, there is a need for large case series-studies with subgroups of various diagnoses and pre-iNO parameters.

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