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ORIGINAL ARTICLE

ROLE OF OXIDATIVE STRESS AND ENDOTHELIAL PRODUCTS IN NEONATAL RESPIRATORY DISTRESS DISORDERS

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Background: *The role of oxidative stresses in the development of endothelial dysfunction and the pathogenesis of pulmonary disorders is probably through alterations in endothelin-1 (ET-1) and nitric oxide (NO) signaling pathways. Free radicals were implicated in the pathogenesis of respiratory distress (RD) in preterm.*

Objective: *To investigate the significance of ET-1, NO, and antioxidant material (ascorbic acid) in newborns with RD and evaluate the effect of other risk factors on its balance.*

Subjects and methods: *Thirty newborns with respiratory distress (group I), and ten healthy newborns as a control group (group II) were enrolled and subjected to routine laboratory and radiological investigations. Serum NO and plasma ascorbic acid were measured on the first day of admission while the plasma ET-1 were measured on first day and seventh day.*

Results: *ET-1 levels were significantly higher in RD group compared to control and in preterm compared to full term infants with RD. ET-1 significantly declined from the first to the seventh day in newborns with RD. Plasma ET-1 was significantly lower in survivors compared to non survivors in the first day of life.*

Conclusion: *Increased vascular resistance in newborns with RD may be related to high ET-1 levels, justifying the use of NO inhalation and recommending trials of new therapeutic strategies, such as endothelin receptor blockers in neonatal RD. Oxidative effect of ascorbic acid may be implicated in the pathogenesis of lung injury in neonatal RD.*

Keywords: *Respiratory distress, Endothelin, Nitric oxide, antioxidant, ascorbic acid, Neonate.*

INTRODUCTION

Pathogenesis of respiratory distress (RD) disorders is complex and involves a variety of oxidative insults which play extremely important role in injury process.[1] Inflammation of the lung may result in activation of macrophages and neutrophils, and release of free radicals due to respiratory burst. In addition, commonly used high inspiratory concentrations of oxygen may contribute to generation of free radicals. Accordingly, a large number of authors have provided evidence that oxidative-degradation products are elevated in acute respiratory distress syndrome (ARDS) patients, both in bronchoalveolar lavage (BAL) and in exhaled breath condensate.[2] This imbalance between newly generated oxidative compounds and the local antioxidative systems may well contribute to lung injury, resulting in increased capillary leakage, altered surfactant metabolism and diminished surfactant function.[3]

An increasing number of studies implicate oxidative stress in the development of endothelial dysfunction and the pathogenesis of pulmonary disorders. This oxidative stress has been shown to be associated with alterations in both the Endothelin-1 (ET-1) and Nitric Oxide (NO) signaling pathways.[4] Recent studies evaluated the role of plasma ET-1 in infants with respiratory distress disorders, and suggested that ET-1 concentration can be used as a useful tool in differential diagnosis between the different respiratory distress disorders.[5]

Endothelin-1 (ET-1), a potent vasoactive peptide released during injurious stimuli, has been reported to increase pulmonary microvascular pressure and lung edema formation via endothelin receptors type A (ETA) and type B (ETB).[6] ET-1 is increased in serum and bronchoalveolar lavage of patients with acute lung injury, suggesting lung endothelial–epithelial dysfunction. The endothelium as a source of oxidative injury releases nitric oxide (NO) via endothelial ETB receptor activation.[7]

Nitric Oxide (NO) is a potent endogenous vasodilator and is involved in cytotoxicity, neurotransmission and immunological defense mechanisms. NO plays an important role in acute lung injury (ALI), acute respiratory distress syndrome (ARDS), and in ventilator-induced lung injury (VILI). A change in the balance of ET-1 and NO in the ALI or ARDS can also add to these problems.[8] Although a role of NO in the regulation of pulmonary blood flow at birth is established, known effects of NO on transcription factors, apoptosis and cellular proliferation, plus the time and spatial limits of pulmonary NO expression, suggest that NO might influence lung development.[1]

Free radicals have been implicated in the pathogenesis of some respiratory disorders in preterm infants. Vitamin C or ascorbic acid is a powerful anti-oxidant in human plasma. Premature infants are born with high levels of plasma ascorbic acid, which decline soon after birth. Some researchers reported that ascorbic acid can act as either anti-oxidant or a pro oxidant depending on its concentration and the presence of metal ions. The plasma levels of ascorbic acid among infants with respiratory distress syndrome are variable.[9]

This study aimed to evaluate the pathophysiological significance of ET-1, NO and antioxidant material (ascorbic acid) in neonates with respiratory distress disorders and to investigate its relation to different risk factors in neonates.

SUBJECTS AND METHODS

Study design: This case - control study was carried out in the Pediatrics and Clinical Pathology Departments, Zagazig University Hospitals from November 2010 to July 2011. Subjects: Thirty newborns with symptoms and signs suggestive of respiratory distress disorders (13 females, 17 males) and 10 healthy newborns as a control group were included in the study. Patients were randomly selected from those admitted to NICU within their first 24 hours after birth, with requirement for supplemental oxygen because of respiratory distress. This group included both full term and preterm newborns. Subjects of the control group were randomly selected from post natal ward.

Newborns were diagnosed as having respiratory distress disorder if they had: Increasing respiratory rate, Chest retraction; intercostal, subcostal and xiphoid, working ala nasi, expiratory grunting, frothing at lips or cyanosis.[10] An informed consent were taken from the next of kens of all cases and controls.

Patients were excluded from the present study if they had any of the following criteria: infection confirmed by cultures, congenital heart diseases, anatomical cause of pulmonary hypertension, progressive intraventricular hemorrhage, lethal congenital anomalies, or severe birth trauma and maternal history of usage of drugs that may affect respiration e.g. sedative.

Methods:

Clinical assessment: Both index and control groups were subjected to full history taking and full clinical examination including determination of the degree of respiratory distress according to Greenough et al.[10] and assessment of gestational age using Ballard score.[11]

Laboratory Assessment:

The following investigations were done for all the included participants:

A. Plain x-ray chest.
B. Routine investigations: Complete blood count (CBC), urine analysis, glucose level, renal function test, liver function test, serum electrolytes and serum calcium and arterial blood gases and bicarbonate.
C. Estimation of level of serum nitric oxide.
D. Estimation of plasma endothelin-1 level using enzyme immune assay (EIA) technique.
E. Estimation of plasma ascorbic acid using electrochemical detection.

All routine laboratory investigations as well as serum nitric oxide, plasma ascorbic acid and x-ray chest were done on the first day of admission while the plasma endothelin-1 was done on first day and one week later.

Specific Laboratory Investigations:

1. Measurement of Serum Nitric Oxide:

Serum nitrate and nitrite is an index of in vivo nitric oxide (NO) generation .So we can measure nitrate level in this study. Nitric oxide was determined by method based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. The reaction is followed by colorimetric detection of nitrite as an azo dye product of the Griess reaction. The Griess reaction is based on the two-step diazotization reaction in which acidified NO2 - produces a nitrosating agent, which reacts with sulfanilic acid to produce the diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylene diamine to form the chromophoric azo-derivative which absorbs light at wave length 540 nm[12] A linear standard curve was generated by plotting the mean absorbance for each standard versus the total nitrite concentration. The kit reagents were supplied form R & D systems (Minneapolis, USA). Results were expressed as µmol/L.

2. Measurement of Ascorbic Acid:

Estimation of ascorbic acid in plasma was estimated using Abcam's Ascorbic Acid Assay Kit (abcam®). Procedure was done according to manufacturer's description. Principle of the assay depends on reduction of Fe3+ to Fe2+ by any antioxidants present. The ferrous iron is chelated with a colorimetric probe to produce a product with a strong absorbance band which was monitored at 545 nm. The addition of ascorbic acid oxidase to parallel samples removes any ascorbic acid present leaving a background value which is subtracted from the total to give ascorbic acid content. Results were expressed as µmol/l..

3. Measurement of Plasma Endothelin-1 Level:

For the analysis of ET-1 we used Human Endothelin-1 Immunoassay KIT For the quantitative determination of Endothelin-1 (ET-1) for serum, EDTA ,heparin and citrated plasma samples. (R&D Systems, Minneapolis, USA). Steps were followed according to the manufacturer recommendations.

This assay employs the quantitative enzyme immunoassay technique. An antibody specific for ET-1 has been pre-coated onto a microplate. Standards, samples, Control and Conjugate are pipetted into the wells and any ET-1 present is sandwiched by the immobilized antibody and the enzyme-linked antibody specific for ET-1. Following a wash to remove any unbound substances and/or antibody-enzyme reagent, substrate is added to the wells and color develops in proportion to the amount of ET-1 bound. The color development is stopped and the intensity of the color is measured at 450 nm. Correction was done at 620 nm.

A standard curve was constructed by plotting the mean absorbance for each Standard on the y-axis against the concentration on the x-axis and a best fit curve through the points was drawn on the graph.

The concentration of each unknown sample was determined by calculating the concentration of ET-1 corresponding to the mean absorbance from the standard curve. According to the manufacturer the minimum detectable level of ET-1 is less than 1.0 pg/ml.

Statistical analysis: Data was collected, entered and checked to an Epi-Info file. Data was expressed as mean Standard Deviation (SD) in quantitative variables, number and percentage for qualitative variables, student t-test, Chi square and correlation coefficient were used for analysis of data, Epi-Info (version 6.02) computer package.[13]

RESULTS

Table 1. Demographic Characteristics of Newborns with Respiratory Distress (group I) Versus Controls (group II).

Characteristics	Group I N = 30		Group II n = 10		Test		Sig.
Sex (no & %)							
Male	17	56.7%	5	50.0%	P		NS
Female	13	43.3%	5	50.0%	0.73		
Age (hrs)							
$\overline{X} \pm SD$	14.7 ± 6.8		17.8 ± 5.9		.		NS
Range	2 – 24		8 – 24		19		
Birth weight (gm)							
$\overline{X} \pm SD$	2800 ± 820		2900 ± 360		0.7		NS
Range	1200 – 4400		2400 – 3500				
Gestational age (weeks)							
$\overline{X} \pm SD$	36.2 ± 3.6		37.5 ± 1.8		0.29		NS
Range	30 – 40		34 – 40				
Risk pregnancies (no & %)							
Yes	9	27%	2	20%	$\chi 2$	P	NS
No	21	73%	8	80%	0.04	0.8	
Apgar score							
1 minute							
$\overline{X} \pm SD$	3.6 ± 1.0		7.4 ± 0.9		t	P	HS
Range	2 – 6		7 – 9		6.6	<0.001	
5 minutes							
$\overline{X} \pm SD$	6.7± 0.9		8.5± 0.8		4.97	<0.001	HS
Range	5 – 8		8 – 10				

$\overline{X} \pm SD$: Mean± standard deviations, **NS** = Non-Significant (P>0.05), **S** = Significant (P<0.05), **HS** = Highly Significant (P<0.001).

Table 2. Clinical Diagnoses in Newborns with Respiratory Distress Disorders (group I).

Diagnosis	No	%
Respiratory Distress Syndrome	13	43.3
Transient Tachypnea of Newborn	8	26.7
Meconium Aspiration Syndrome	6	20.0
Persistent Pulmonary Hypertension	3	10.0

Table 3. Mean ± SD of Plasma Levels of ET-1, NO and Ascrobate in Studied Groups.

Characteristics	Group I n = 30	Group II n = 10	t test	P Value	Sig.
ET-1* (pg/ml)					
Day 1					
$\overline{X} \pm SD$	11.1 ± 3.85	1.24 ± 0.42	7.98	<0.001	HS
Range	3.8 – 18.73	0.57 – 1.92			
Day 7					
$\overline{X} \pm SD$	4.2± 2.37				
Range	0.9 - 10.7				
NO** (µmol/L)					
$\overline{X} \pm SD$	18.4 ± 8.0	47.9 ± 8.2	10.02	<0.001	HS
Range	5.3 – 50.8	35.6 – 59.5			
Ascorbic acid ***(µmol/L)					
$\overline{X} \pm SD$	49.0 ± 17.2	35.8 ± 11.1	2.25	0.028	S
Range	19.6 – 79.5	23.4 ± 57.8			

$\overline{X} \pm SD$: Mean± standard deviations, **NS** = Non-Significant (P>0.05), **S** = Significant (P<0.05), **HS** = Highly Significant (P<0.001).

Table 4. Comparison between the degrees of respiratory distress and ET-1, NO and ascorbic acid.

Degree \ Item	ET-1	NO	Ascorbic acid
Mild – Moderate (n=21)			
$\overline{X} \pm SD$	11.3 ± 3.8	18.1 ± 4.4	46 ± 16.7
Range	3.8 – 18.75	7.6 – 27.5	19.6 – 72.3
Severe – Advanced (n=9)			
$\overline{X} \pm SD$	11.15 ± 4	19.2 ± 13.5	56.1 ± 17.3
Range	5.8 – 18.3	5.3 – 50.8	33.6 -79.5
T test	0.07	0.34	1.4
P value	0.93 NS	0.73 NS	0.14 NS

$\overline{X} \pm SD$: Mean± standard deviations, **NS** = Non-Significant (P>0.05), **S** = Significant (P<0.05), **HS** = Highly Significant (P<0.001).

Table 5. Correlation between ET-1, NO and Ascorbic Acid with Weight and Gestational Age.

Item	Weight		Significance	Gestational Age		Significance
	r	p		r	p	
ET–1	0.15	>0.05	NS	0.39	<0.05	S
NO	0.11	>0.05	NS	-0.12	>0.05	NS
Ascorbic acid	-0.58	<0.001	HS	-0.64	<0.001	HS

NS = Non-Significant (P>0.05), **S** = Significant (P<0.05), **HS** = Highly Significant (P<0.001).

Table 6. Correlation between both ET-1, NO and Ascorbic Acid with Apgar Score at 5 Minutes.

Item \ Score	Apgar Score		Significance
	r	P	
ET–1	- 0.56	0.001	HS
NO	+ 0.63	0.001	HS
Ascorbic acid	- 0.31	0.05	S

NS = Non-Significant (P>0.05), **S** = Significant (P<0.05), **HS** = Highly Significant (P<0.001).

Table 7. Relation between Mean ± Standard Deviation of NO, ET-1 and Ascorbic Acid Levels and Outcome in Both Groups.

	Survival N = 23	Mortality N = 7	t test	P Value
NO level				
$\overline{X} \pm SD$	18.9 ± 8.3	16.7 ± 7.1	0.54	NS
Range	7.6 – 50.8	5.3 – 24.9		
ET-1 level				
Day 1				
$\overline{X} \pm SD$	10.3 ± 3.2	13.6 ± 4.97	2.13	0.03*
Range	3.8 – 15.2	5.8 – 18.73		S
Day 7				
$\overline{X} \pm SD$	3.98 ± 2.3	5.9 ± 2.4	1.3	>0.9
Range	0.9 – 10.7	3.12 – 7.45		NS
Ascorbic acid level				
$\overline{X} \pm SD$	46.6 ± 18.1	57.1 ± 11.9	1.45	0.156
Range	19.6 – 79.5	44.3 – 76.8		NS

$\overline{X} \pm SD$: Mean± standard deviations, **NS** = Non-Significant (P>0.05), **S** = Significant (P<0.05), **HS** = Highly Significant (P<0.001).

Table 8. Correlation Matrix Between Level of ET-1, NO and Ascorbic Acid.

	ET–1				NO		Ascorbic acid	
	Day1		Day 7					
	r	P	r	P	r	p	r	P
ET-1 Day 7	0.51	<0.001 HS						
NO	0.13	>0.05 NS						
Ascorbic acid	0.16	>0.05 NS	<0.001	>0.05 NS	0.26	>0.05		

NS = Non-Significant (P>0.05), **S** = Significant (P<0.05), **HS**= Highly Significant (P<0.001).

DISCUSSION

The present study investigated levels of ET-1, NO, and ascorbic acid as an antioxidant in newborns with respiratory distress, and the effect of other risk factors i.e. prematurity, birth weight, and mode of birth on them.

Thirty newborns suffering from Respiratory Distress with different etiologies were enrolled in this study. A group of 10 healthy full term neonates served as a control group.

ET-1 levels were significantly higher in newborn infants with respiratory distress disorders compared to control group (11.1 + 3.85 pg/ml versus1.24+0.42 pg/ml) (Table 3). This is in agreement with the results of the experimental study of de Vroomen et al.[14] which proved a highly significant increases in plasma ET-1 level in early phase of pulmonary hypertension development, during RD in newborn lambs. Increased ET-1 concentration during respiratory distress appeared to be reached in the early phase of pulmonary hypertension development.[14]

Kuo et al.[15] found variable levels of ET-1 in newborns suffering respiratory distress of different etiologies, however they could not conclude whether ET-1 can be considered as a marker or a mediator of pulmonary hypertension in newborns with RDS or not. Our results also agreed with those of Benzer et al.[16] and El Sayed et al.[17] who found out that plasma ET-1 concentration in newborns with RDS were higher compared to the newborns without RDS.

In the present study, the increased circulating ET-1 level was not significantly correlated with the severity of respiratory distress assessed according to Greenough[10] and Silverman[18] as shown in (Table 4). This is in agreement with Niu et al.[19], who reported a high level of ET-1 in airways of preterm infant and it was not related with the severity of respiratory distress during the early postnatal period. In contrast, de Vroomen et al., [14] found a correlation between the severity of the distress and levels of ET-1. Kumar et al.[20] showed that the presence of elevated ET-1 concentrations were positively correlated with disease severity and suggested that ET-1 may serve as a marker of the disease severity in these infants. This discrepancy between our results and others may be explained by the different etiologies of RDS in the selected groups, and may require a study with larger number of patients and targeted specific etiologies of RD.

In the present study, there was no statistically significant relation between the mode of delivery and level of ET-1 in patients with respiratory distress in the first day and 7th day of life (11.6+ 3.8 pg/ml in day1 and 3.9 + 2.2 pg/ml in day7 for SVD) in comparison with (10.6+ 3.96 in day 1 and 4.4+ 2.5 in day7 in CS). This result is in coincidence with Niu et al.[19] and Endo et al.[21] who found no significant differences in serum ET-1 and NO concentrations between the spontaneous labor group and the elective cesarean group at the birth.

These results were not in coincidence with other investigators who reported a higher occurrence of respiratory morbidity in late preterm and term infants delivered by elective cesarean section. Those infants have a higher incidence of TTN, RDS and severe PPHN.[22] Some of these reports also showed higher rates of NICU admission, mechanical ventilation, oxygen therapy and even death.[23]

However, in day 5 Endo et al.,[21] observed that ET-1 and NO concentrations were significantly higher in the SVD group than in elective cesarean group. So it was speculated that spontaneous labor might enhance endogenous NO synthesis at 5th day of age. This can be explained by the fact that biochemical and hormonal changes that accompany vaginal delivery play an important role in fetal pulmonary transition. For effective gas exchange to occur, alveolar spaces must be cleared of excess fluid and ventilated, and pulmonary blood flow increased to match ventilation and perfusion. Failure of either of these events can jeopardize neonatal transition and cause infants to develop respiratory distress.[24]

In our study ET-1 was significantly higher in preterm newborns compared to full term newborns with RD disorders as shown in (Table 5). This finding was in agreement with many investigators.[25,26] Fagan et al. explained the role of ET-1 in developing RD by its vasoconstrictor, mitogenic, and inflammatory actions.[26] In disagreement with our results, Benzing et al[27] stated that Plasma concentrations of the stable endothelin-1 precursor, determined prospectively in 293 newborn infants (gestational age 24-41 weeks) at birth and on day 3 of life were unrelated to gestational age at birth, but strongly associated with respiratory distress when measured on day 3 of life.

Respiratory distress syndrome of premature infants is caused by a structural immaturity of lungs and insufficient production of surfactant.[1] Preterm infants with respiratory distress very often suffer from periods of hypoxia during the course of the disease that may stimulate ET-1 production.[28]

In the present study, high plasma ET-1 levels in the first day of life were associated with lowered Apgar score (Table 6) and elevated mortality rate of newborns with respiratory distress disorders (Table 7). Plasma ET-1 concentrations may be a good indicator of prognosis since the first day of life after birth. It was significantly different between survivors and non-survivor newborns, in whom

the ET-1 concentrations were higher in the first day of life. This result is in agreement with Benzer et al.[16] who suggested that those newborns might suffer severe damage in their lungs, starting from the first few hours after birth. In contrary, Benjamin et al.[5] did not observe any association between high plasma ET-1 levels and increased mortality in infants with BPD , a finding which can be addressed in the view of BPD being single and chronic reason for RD.

In our study we found that newborn infants with respiratory distress disorders had significantly lower plasma concentration of NO than in control group. This result runs in agreement with many other investigators who reported that either NO or its precursor; arginine, are lower in newborns infants with respiratory distress disorders.[29-31]

In the present study no significant correlation was observed between the level of NO and the degree of respiratory distress, a result which is in contrast to that of Sirven et al.[32] who reported in his study that neonates with BPD and RDS, had levels of NO that coincided with disease severity. This discrepancy may be explained by the difference in etiologies of RD in the studied groups and the use of other surrogate markers for assessment of the disease severity and inflammation in his study.

However, in the present study some other clinical and laboratory parameters which might be used for assessing the disease severity e.g. Apgar score (Table 6) and arterial blood gases were highly significantly related to level of NO. This means that the changes of vasoreactivity effects of NO were associated with more lung damage and more severe category of respiratory distress in newborn. These results are in parallel with Sirven et al.,[32] who reported that the changes in levels of NO coincide with lower Apgar score .

In agreement with Endo et al.,[21] our results showed no significant correlation between the changes in NO levels and mortality in newborn infants with respiratory distress disorders (Table 7). The study showed no statistically significant correlation between prematurity and level of serum NO in newborns with respiratory distress. These changes are not in agreement with other studies [21] [33] [34]. These findings can be explained by the fact that the ability of the endothelium to produce or sustain the production of NO in response to specific stimuli during maturation lags behind the capacity of fetal pulmonary smooth muscle to relax to NO. This may account for the interesting clinical observation that extremely preterm newborns are highly responsive to inhale NO.[35]

In (Table 3), the plasma levels of ascorbic acid were significantly higher in newborns with respiratory distress compared to the control group. This result is in agreement with Reiter and Karbownik[36] and Ciencewick et al.[37] who explained the damage induced by oxygen and its related radical species by two mechanisms, the first is an enzymatic scavenging of the oxygenated radical or chemical scavengers (such as vitamin E, carotenes and other antioxidant). The second defense mechanism involves the removal of oxidized group and repair processes [38]. high level of ascorbic acid which may be present in those newborns since birth and did not start to decline as expected soon after birth may act as a pro-oxidant in these group of newborns causing lung damage and development of respiratory distress of different etiologies.

There are many binding proteins in human plasma which prevent metal ions from participating in free radical reactions such as transferrin and ceruloplasmin. It was found that transferrin and ceruloplasmin levels are low in infants, resulting in higher circulating Fe2+.[39] In addition, the pro-oxidant effect of ascorbate may inhibit ferroxidase activity of ceruloplasmin by reducing cupric (Cu2+) to cuprous (Cu+) so that Fe2+ cannot be oxidized to Fe3+ Therefore, the Fe2+ ion can react with hydrogen peroxide to form a hydroxyl free radical (OH) which is extremely reactive and generates numerous free radicals. The increase in free radicals in infants may lead to complications such as bronchopulmonary dysplasia, necrotizing enterocolitis, retinopathy of pre-maturity, respiratory distress syndrome and intraventricular hemorrhage.[40]

Our results revealed a significant correlation between the ascorbic acid levels and both Apgar score and birth weight (Table 5,6). These results agree with Boonsiri et al.[9] and Falciglia et al.[41] who reported that premature infants with poor outcome had higher levels of ascorbic acid than those with good outcome.

In the present study, although the level of ascorbic acid was much higher in newborns with severe to advanced degree of respiratory distress compared to the newborns with mild to moderate degree of respiratory distress (Table 4), the differences were not statistically significant. The level of ascorbic acid in newborn infants who died was higher than in those who survived. These results are in coincidence with that reported by Boonsiri et al.[9] where infants with poor outcome had a higher concentration of plasma ascorbic acid than those with good outcome. The high ascorbic acid levels in newborn infants with respiratory distress who died may have promoted free radical generation. So ascorbic acid supplementations to newborns with respiratory problems (especially preterm infant) require caution since it may cause poor outcomes.

On studying the correlation matrix between ET-1, NO and ascorbic acid (Table 8), there was a highly significant negative correlation between the elevation of ET-1 levels on the first day of life and the decrease in level of NO. Meanwhile, non-significant correlations were observed between the elevated ET-1 levels and the increase in plasma ascorbic acid levels. On the 7th day of life the level of ET-1 also showed a significant negative correlation with NO level and non-significant correlation with level of ascorbic acid. A non-significant correlation was seen between the changes in NO levels and ascorbic acid.

The limited endogenous NO production and the elevated ET-1 production during the first few days of life may contribute to the vasoactive imbalance in pulmonary transition and circulation in newborn infants which lead to development of respiratory distress disorders in newborn infants.[21] This is in parallel with many other investigators who clarified the relationship between the vasodilator NO with vasoconstrictor ET-1 in causation of different etiologies of respiratory problems in newborns.[21,42,43]

CONCLUSIONS

The increased vascular resistance in newborns with respiratory distress disorder may be related to high ET-1 levels, justifying the use of NO inhalation and recommending future studies and trials of new therapeutic modalities, such as ETA receptors blockers in newborn with respiratory distress disorders. In addition we speculated that plasma ET-1 may be a specific marker for pulmonary endothelium injury in newborns with respiratory distress disorders of different etiologies. Oxidative effect of ascorbic acid may be involved in the pathogenesis of lung injuries in neonatal respiratory distress disorders, suggesting further researches for use of anti-oxidant as a treatment modality and indicating cautious administration of ascorbic acid in neonates.

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