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Endothelin 1 As A Predictor Marker for Bronchopulmonary Dysplasia in Preterm Neonates with Respiratory Distress Syndrome

Hanaa Abd Elftah Mohamed[1], Yousef Mohamed Yosef[1], Noha Abd Elhaleem Mohamed[2], Amal Abdo Ahmed Alsyed[1]

Department of [1]Pediatrics and [2]Biochemistry, Faculty of Medicine, Zagazig University, Egypt

***Corresponding author:** Amal Abdo Ahmed Alsyed, **Mobile:** (+20) 01288778806, **E-Mail:** dr-amal08@hotmail.com

ABSTRACT

Background: Bronchopulmonary dysplasia (BPD) is usually defined as a need for supplemental oxygen at 36 weeks after conception. Functional data on endothelin-1 action suggests that endothelin-1 is not only a marker but also a mediator of respiratory disease in newborn infants including BPD.

Objective: The aim of this study was to evaluate the value of endothiline-1 as a biomarker that predicts early diagnosis of bronchopulmonary dysplasia in preterm neonates with respiratory distress syndrome (RDS).

Patients and Methods: This study was conducted as prospective study in Neonatal Intensive Care Unit, Zagazig University Hospital, Zagazig, Egypt in the period between April 2018 and October 2018. The study included 39 preterm neonates, (22 males and 17 females) with gestational age of 28-35 weeks who were diagnosed as having respiratory distress syndrome based on clinical and radiological findings.

Results: In the present study, cases group had significant lower gestational age 30.22 ± 1.81 week & birth weight 1.35 ± 0.22 Kg compared to the control group 34.58 ± 2.19 weeks & 1.69 ± 0.38 Kg respectively. In the present study, there was no significant difference between study and control groups as regards Apgar score at 1 & 5 minutes, gender and mode of delivery. Also, serum endothelin-1 level was done at day 3 of postnatal life and it was significantly higher in neonates who developed BPD later in life (435.29 ± 172.83 ng/l) compared to the control group with no BPD (304.32 ± 54.46 ng/l). Neutrophil count on day 3 of life was positively correlated with level of endothelin-1.

Conclusion: Our findings indicated that high serum levels of endothelin-1 at day 3 of life was associated with later development of bronchopulmonary dysplasia.

Keywords: Endothelin-1, Predictor marker, Bronchopulmonary dysplasia, Preterm neonates, respiratory distress syndrome.

INTRODUCTION

Prematurity refers to the broad category of neonates born at less than 37 weeks' gestation, although the estimated date of confinement (EDC) is 40 weeks' gestation, the World Health Organization (WHO) broadened the range of full term to include 37-42 weeks' gestation. Serious morbidities occur in preterm infants include respiratory distress syndrome and bronchopulmonary dysplasia. In the general population, 12% of infants are born prematurely **(1).**

Respiratory distress syndrome, also known as hyaline membrane disease, occurs almost exclusively in premature infants, the incidence and severity of respiratory distress syndrome are related inversely to the gestational age of the newborn infant **(2)**.

NICHD has defined and adapted the criteria for BPD as need of oxygen support (> 21%) at 36 weeks of postmenstrual age (PMA) in infants born < 32 weeks while need of supplemental oxygen for > 28 days but < 56 days postnatal age on infants born > 32 weeks **(3).**

Bronchopulmonary dysplasia is a form of chronic lung disease that develops in preterm neonates treated with oxygen and positive pressure ventilation. The pathogenesis of this condition remains complex and poorly understood. Models have been developed for predicting the probability of BPD at specific postnatal time points using readily available clinical data **(4).**

Endothelin (ET) is a potent contracting factor that was isolated in pulmonary and systemic endothelial cells in the mid-1980 and it was eventually characterized in 1988 as a 21-amino acid peptide. ET was soon defined as the most potent and long-lasting endogenous vasoconstrictive substance yet discovered. Since then the ET system has been found to be involved in multiple physiologic functions related to the respiratory, nervous, renal, cardiovascular, gastrointestinal and endocrine systems. In addition, ET system seems to be implicated in many disease states including bronchoconstriction, fibrosis, and pulmonary hypertension **(5).** The aim of this study was to evaluate the value of endothiline-1 as a biomarker that predicts early diagnosis of bronchopulmonary dysplasia in preterm neonates with respiratory distress syndrome.

PATIENTS AND METHODS

This study was conducted as prospective study in Neonatal Intensive Care Unit, Zagazig University Hospital, Zagazig, Egypt in the period between April 2018 and October 2018. The study included 39 preterm neonates, (22 males and 17 females) with gestational age ranged from 28 to 35 weeks and diagnosed as having respiratory distress syndrome based on clinical and radiological findings. They were divided into 2 groups: **1-The study (BPD) group:** It included 17 preterm neonates who developed bronchopulmonary dysplasia. **2-The control (Non-BPD) group:** It included 22 preterm neonates who did not develop bronchopulmonary dysplasia. The premature baby was diagnosed as a baby born before 37 weeks of gestation or with fewer than 259 days of gestation**.**

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Exclusion criteria: Major congenital malformations, chromosomal aberrations, inborn errors of metabolism, and other types of pneumonia.

Respiratory Distress Syndrome was diagnosed clinically based on the presence of respiratory distress (tachypnea, intercostal retractions, and/or nasal flaring) shortly after delivery and a persistent need for respiratory support (oxygen or positive pressure) for more than 24 hours with a typical chest radiograph (reticular granular appearance of pulmonary parenchyma with air bronchograms progressing up to ground glass appearance) [6]. Spectrum of chest X-ray ranged from mild to severe, which was generally correlated with the severity of the clinical findings [7].

Bronchopulmonary dysplasia (BPD) was diagnosed based on:

Clinically: (1) For preterm neonates > 32 weeks: a need for supplemental oxygen > 28 days post-natal. (2) For preterm neonates < 32 weeks: a need for supplemental oxygen at 36 weeks after conception.

Radiologically by chest X-ray: Gradual change from complete opacification with air bronchogram & interstitial emphysema to small round radiolucent areas alternating with irregular density resembling sponge.

All neonates included in the study were subjected to the following:

1- Full history taking laying stress on: History of PROM > 18 hours, previous abortion, parity, gestational age, mode of delivery, anti-natal steroids, prepartum problems (UTI/ vaginal bleeds, any medication, fever, rash, PIH, anemia, diabetes, GDM, abnormal presentation and USG findings), and maternal sepsis.

2- Complete clinical assessment laying stress on: Gestational age assessment using new Ballard score [8], birth weight, sex, and complete heart, chest, abdominal & neurological examination.

3- Recorded Apgar score at 1 minute and 5 minutes.

4- Laboratory investigations: (a) CBC at day 1 and day 3 of life. (b) CRP with titer at day 1 and day 3 of life. (c) Blood culture & sensitivity using Bactec system showed type of organism & antibiotic sensitivity. (d) Serum human endothelin-1 done at day 3 of life (using kit no. MBS294582) by ELISA technique

5- Chest X-rays: for diagnosis of RDS on admission & repeated when required & on diagnosis of BPD.

6- Follow-up of neonates till discharge to determine: (a) Use of surfactant. (b) Days on NCPAP. (c) Days on mechanical ventilator. (d) Days on other oxygen therapy. (e) Days of hospital stay. (f) Mortality.

Specimen collection and preparation:

1. **CBC** with differential leucocytic count using CELL DYN 1800 hematology analyzer was done for preterm neonates of both groups at day 1 and day 3 postnatal life.
2. **C-reactive protein (CRP)** quantitative assay using latex agglutination test for preterm neonates of both groups was done at day 1 and day 3 postnatal life.
3. **Blood culture** using Bactec system for preterm neonates of both groups was done at day 1 postnatal life, 2 cc of blood were withdrawn.
4. **Serum human endothelin-1** was done at day 3 of life using ELISA technique: Allow the sample to clot for 10-20 minutes at room temperature, then place in centrifuge (at 2000-3000 RPM) for approximately 20 minutes. Collect the supernatants carefully.

Principle:

This kit used enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay human endothelin-1 (ET-1). Endothelin-1 (ET-1) was added to wells that were pre-coated with endothelin-1 (ET-1) monoclonal antibody and then was incubated. After incubation, anti ET-1 antibodies labeled with biotin was added to unite with streptavidin-HRP, which formed the immune complex. Unbound enzymes removed after incubation and washing, and then substrates A and B were added. The solution turned blue and changed to yellow with the effect of acid. The shades of solution and the concentration of human endothelin-1 (ET-1) were positively correlated.

Ethical consent:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. All neonates were included after an informed consent taken from their parents. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistics and analysis

The collected data were revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 20). Data were presented and suitable analysis was done according to the type of data obtained for each parameter. Mean, standard deviation (± SD) and range were used for parametric numerical data, while median and interquartile range (IQR) were used for non-parametric numerical data. Frequency and percentage for non-numerical data. Student t Test was used to assess the statistical significance of the difference between two study group means. Correlation analysis (using Pearson's method) was used to assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "r" defines the strength and direction of the linear relationship between two variables. Chi-Square test was used to examine the relationship between two qualitative variables. Fisher's exact test was used to examine the relationship between two qualitative variables when the expected count is less than 5 in more than 20% of cells. The ROC Curve

(receiver operating characteristic) provides a useful way to evaluate the sensitivity and specificity for quantitative diagnostic measures that categorize cases into one of two groups. Logistic regression was useful in the prediction of the presence or absence of an outcome based on a set of independent variables. It is similar to a linear regression model but is suited when the dependent variable is qualitative (categorical). $P \leq 0.05$ was significant.

RESULTS

Table (1) showed the maternal characteristics of cases.

Table (1): The maternal characteristics of cases

		Case (n=39)	
Quantitative		**Mean**	**SD**
Age (years)		29.53	1.81
Qualitative		**N**	**%**
Previous Abortion	**Yes**	17	43.5
	No	9	32.07
Rh Blood Groups	**Rh+**	31	79.4
	Rh-	8	20.5
Parity	**Primiparous**	21	53.8
	Multiparous	18	48.7
ABO Blood Group	**O**	17	43.5
	A	9	32.5
	B	8	20.5
	AB	5	12.8
Previous Medications	**Yes**	11	28.2
	No	28	71.7
Prepartum problems[1]	**1st Trimester**	1	2.5
	2nd Trimester	2	5.12
	3rd Trimester	3	7.6
	No	33	84.6
PROM>18	**Yes**	7	17.9
	No	29	74.3
Maternal sepsis	**Yes**	1	2.5
	No	38	97.4
Antenatal steroids	**Yes**	30	
	No	9	

[1]: Prepartum problems include:

First Trimester- UTI/ vaginal bleeds/ any medication/ fever or/and rash. Second Trimester- Fever and/or rash, PIH, Medications, anaemia, diabetes. Third Trimester- PIH/GDM/abnormal presentation/USG findings/ medications.

There were significant differences considering average G.A. and B.W being higher in control group. Moreover, no significant difference was found between both groups as regards Apgar score at 1 and 5 minutes, sex and mode of delivery. Moreover, all neonates were born as a single (i.e. no twin cases have been recorded) in both groups (Table 2).

Table (2): Comparison of the neonatal quantitative and qualitative characteristics between cases (RDS with BPD) and controls (RDS)

		Case group [RDS with BPD] (n=17)		Control group [RDS] (n=22)		P-value	Sig
		Mean	**SD**	**Mean**	**SD**		
GA (Wks)		30.22	1.81	34.58	2.19	0.001*	HS
BW (Kg)		1.35	0.22	1.69	0.38	0.027*	S
Apgar 1 (Min)		4.71	1.05	4.77	1.27	0.862	NS
Apgar 5 (Min)		8.24	0.66	8.32	1.43	0.811	NS
		N	**%**	**N**	**%**		
Sex	**Female**	8	47.1%	9	40.9%	**0.732**	**NS**
	Male	9	52.9%	13	59.1%		
MOD	**CS**	11	64.7%	12	54.5%	**0.52**	**NS**
	SVD	6	35.3%	10	45.5%		
Single		17	100.0%	22	100.0%	-	-

GA=Gestational age. Wks=Weeks. BW=Birth weight. Min=Minutes. SD=standard deviation. Sig=Significance. HS=highly significant. NS=Non-significant. MOD=mode of delivery. CS=Cesarean section. SVD=Spontaneous vaginal delivery.

Considering ABG findings, the percentage of neonates suffering from hypoxia and acidosis was significantly higher in the cases than controls. However, X-ray findings showed higher percentage of severe lung lesions within BPD neonates compared to the controls. As mentioned before that the overall percentage of neonates on mechanical ventilation was significantly higher in the BPD than in the controls. The most frequently used mechanical ventilation modes were SIMV followed by CPAP. The percentage of BPD neonates on SIMV was more than twice the percentage of control neonates using SIMV (Table 3).

Table (3): Comparison of grading of RDS characteristics and the mode of O_2 admission between cases (RDS with BPD) and controls (RDS)

		Case group [RDS with BPD] (n=17)		Control group [RDS] (n=22)		P- value	Sig
		N	%	N	%		
ABG	**Acidosis-hypoxia**	12	70.59	5	22.7	< 0.0001	HS
	Normal	5	29.41	17	77.3		
X-ray diagnosis	**Mild**	1	5.88	10	45.5	< 0.0001	HS
	Moderate	2	11.76	8	36.4		
	Severe	14	82.35	4	18.2		
SIMV		6	35.29	3	13.6	< 0.0001	HS
CPAP		3	17.65	5	22.7		
Other O_2 therapy		8	47.06	14	63.6		

ABG = arterial blood gas. HS= highly significant. SIMV=Synchronized intermittent mandatory ventilation. CPAP=Continuous positive airway pressure. SD=standard deviation.

Table (4) showed that endothelin-1 done at day 3 of life was significantly higher in neonates who developed BPD (cases group) compared to those who didn't develop BPD (control group).

Table (4): Comparison of endothelin-1 levels between cases (RDS with BPD) and control groups (RDS) at the 3rd day of life

	Case group [RDS with BPD] (n=17)		Control group [RDS] (n=22)		p- value	Sig.
	Mean	**SD**	**Mean**	**SD**		
Endothelin-1(ng/l)	435.29	17.83	304.32	54.46	0.007	HS

HS=highly significant

Table (5) showed that neutrophil count at day 3 of life was significantly positively correlated with endothelin-1 while there was no significant correlation between endothelin-1 and other laboratory parameters.

Also, there was no significant correlation between endothelin-1 and gestational age or birth weight. Additionally, there was significant correlation between levels of endothelin-1 and days on SIMV and highly significant correlation with days on CPAP.

The higher the level of endothelin-1 the more days the patient spent on SIMV and on CPAP. However, there was no significant correlation between levels of endothelin-1 and days on other O_2 therapy.

Table (5): Correlation between endothelin-1 and different parameters

	Endothelin-1		
	Pearson correlation	**P-Value**	**Sig**
GA	-0.188	0.253	NS
BW	-0.21	0.2	NS
WBC D.1	0.151	0.36	NS
Neut D.1	0.065	0.696	NS
CRP D.1	0.051	0.757	NS
WBC D.3	0.203	0.215	NS
Neut D.3	0.347	0.03	S
CRP D.3	0.028	0.864	NS
Days on SIMV	0.387*	0.015	S
Days on CPAP	0.578**	<0.001	HS
Days on nasal O_2	0.076*	0.645	NS
Days on O_2 therapy	0.401*	<0.001	HS

GA=Gestational age. BW=Birth weight. WBC=white cell count. Neut=neutrophils. CRP=C – Reactive protein. D.1=Day one of life. D.3=Day three of life. S=Significant. NS=Non-significant. SIMV=Synchronized intermittent mandatory ventilation. CPAP=Continuous positive airway pressure. HS=highly significant.

When we used the ROC curve to predict the occurrence of BPD by using endothelin-1 as a marker, we found that it had highly significance as the optimal cutoff point (i.e. that corresponds to highest sensitivity and specificity) was 295.5 with 94% sensitive and 73% specificity in predicting BPD (Table 6).

Table (6): Interpretation of ROC curve for using endothelin-1 to predict bronchopulmonary dysplasia

AUC	0.809
SE	0.068
Cut off point	295.5
Sensitivity	0.94
Specificity	0.73
C.I. (95%)	0.676 - 0.942
Sig	< 0.001

AUC=Area under curve. SE=Standard error. C.I. =Confidence interval. Sig=Significance.

Table (7) showed that when we used the ROC curve to predict mortality in BPD group by using endothelin-1 as a marker we found that it had no significance as the optimal cutoff point (i.e. that corresponds to highest sensitivity and specificity) was 307.5 with **1**% sensitivity and 33% specificity.

Table (7): Interpretation of ROC curve for using endothelin-1 to predict mortality in BPD group

	Endothelin-1
AUC	0.514
SE	0.15
Cut off point	307.5
Sensitivity	1
Specificity	0.33
C.I. (95%)	0.219 - 0.808
Sig	0.923

AUC=Area under curve. SE=Standard error. C.I. =Confidence interval. Sig=Significance.

DISCUSSION

In our study, the frequency of BPD among the studied patients was 43%, more than that mentioned by **Tapia *et al.*** [9] who found that the incidence of BPD was 24.4% in a large South American population. A possible explanation is that in our study we had a higher mean gestational age 30.5 ± 1.81 weeks and birth weight 1.27 ± 0.29 Kg compared to **Tapia *et al.*** [9] results where in their study mean gestational age and birth weight were 29 ± 3 weeks and 1.085 ± .279 Kg respectively. **Ehrenkranz *et al.*** [10] found that the incidence of BPD was 52% in infants with birth weights of 501-750 g, 34% in neonates with birth weights of 751-1000 g, 15% in neonates with birth weights of 1001-1200 g, and 7% in neonates with birth weights of 1201-1500 g.

In the present study, there was significant lower gestational age (30.22 ±1.81 weeks) & birth weight (1.35 ± 0.22 Kg) in the cases group compared to the control group (34.52 ± 2.19 weeks) & (1.69 ± 0.38 Kg) respectively. In agreement with our results, **El Shemi *et al.*** [11] reported increased risk of BPD with lower gestational age (30.53 ± 1.81 weeks) & lower birth weight (1.27 ± 0.29 Kg). Also, our findings are in agreement with a study mentioned by, **Tapia *et al.*** [9] who reported increased risk of BPD with lower gestational age (29 ± 3 weeks) & lower birth weight (1.085 ± 0.279 Kg). Also, our findings are in agreement with a study mentioned by **Dravet-Gounot *et al.*** [12] who studied 44 preterm neonates. In their study, the neonates who developed BPD had lower gestational age (29.5 ± 2.2 wks) & lower birth weight (1.103 ± 0.237 Kg) compared to control group (32.6 ± 2.2 wks & 1.558 ± 0.468 Kg respectively). Besides, our findings agree with a study done by **Demirel *et al.*** [13] who found that the mean gestational age & birth weight of neonates who developed BPD was 28.98 ± 2.21 weeks &1.154 ± 0.238 Kg while it was 30.0 ± 1.91 weeks & 1.281 ± 0.161 Kg in the control group.

We didn't find significant difference between cases and control as regards gender or mode of delivery. On the contrary of our results, **Hansen *et al.*** [14] found that delivery by elective Cesarean section was associated with an increase of respiratory morbidity. The magnitude of this relative risk seemed to depend on gestational age even in deliveries that completed 37 weeks of gestation. This might be explained by that vaginal delivery stimulate more corticosteroids release in mothers with their subsequent effect in improving outcome of RDS and so decrease incidence of BPD. In agreement with our results, **El Shemi *et al.*** [11] reported that there is no significant difference between sexes in each group. On the contrary of our results, **Tapia *et al.*** [9] observed that female gender was associated with decreased risk of BPD, and also reported lower incidence of Cesarean section in the neonates with BPD.

In the present study, serum endothelin-1 level done at day 3 of postnatal life was significantly higher in neonates who developed BPD later in life compared to the control group with no BPD. In agreement with our results, **Niu *et al.*** [15] compared between tracheal aspirate in preterm infants with RDS who subsequently had BPD, with those who had self-limited RDS, to determine whether ET-1 concentration in tracheal aspirate (TA) could be used as a specific marker for acute lung injury in preterm infants with RDS. The study demonstrated that ET 1 levels in the TA of infants with BPD were significantly higher on day 1 and throughout the first week of life than in the TA of those without BPD. Possible explanation that is ET-1 has been hypothesized to increase the production of oxygen free radicals. It was theorized that in patients with BPD, endothelin-1 would be up-regulated secondary to the inflammatory response during the very early stages of acute lung injury [16]. Also, **Benzing *et al.*** [17] reported that infants in whom BPD developed had higher proET-1 (the stable precursor of ET-1) concentration on day 3 than infants without BPD. On the contrary, **Kuo *et al.*** [18] reported that in RDS infants, endothelin-l concentration had no significant difference between those who developed BPD (7.84 ± 1.85 pg/ml) and those who recovered (5.81 ± 2.76 pg/ml). Also, **Benjamin *et al.*** [19] reported that they did not observe any association between high plasma ET-1 levels and progression to BPD or increased mortality. This distinction may require a study with larger number of patients as they studied only18 preterm neonates with RDS**.** Also, **Kojima *et al.*** [20] reported that no significant difference in endothelin-1 level between cases group of RDS with BPD (18 ± 6 pg/ml) & control group of RDS without BPD (11.7 ± 2.7 pg/ml). Also, they reported that endothelin-1 levels in infants with RDS uncomplicated with BPD gradually decreased in

the 2nd week of life. This might be the explanation as these enothelin-1 levels were measured in the 2nd week of life whereas our samples were taken on day 3 of life.

In the present study, endothelin-1 had higher levels in neonates stayed more days on SIMV or CPAP. While endothelin-1 was not correlated with neonates stayed more days on other O_2 therapy. This might be explained by that neonates with high levels of endothelin-1 had a higher probability to be oxygen-dependent for a long-time and so BPD occurs later in life.

In the present study, risk factors of BPD were lower birth weight, lower gestational age and higher serum endothelin-1 on day 3 of life. In agreement with our results, **Charitharth and Namasivayam** [(21)] demonstrated in their study that one of the major predictors of BPD was lower gestational age. **Tapia *et al.*** [(9)] reported that the risk factors for bronchopulmonary dysplasia in their study were surfactant requirement, mechanical ventilation, air leak, patent ductus arteriosus, late onset sepsis and necrotizing enterocolitis.

In our study, endothelin-1 level at a cutoff point of 295.5 could predict BPD with sensitivity of 94% and specificity of 73%.

CONCLUSION

Our findings indicated that high serum levels of endothelin-1 at day 3 of life was associated with later development of bronchopulmonary dysplasia.

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REFERENCES

1. **Quinn J, Munoz F, Gonik B *et al.* (2016):** Preterm birth: Case definition & guidelines for data collection, analysis, and presentation of immunisation safety data. Vaccine, 34 (49): 6047-6056.
2. **Pramanik A, Donn S, Wanger C *et al.* (2006):** Respiratory distress syndrome. Medicine Journal, 3: 7-12.
3. **Concina V, Samide A, Bada H (2018):** Comparing Diagnostic Criteria for Bronchopulmonary Dysplasia (bpd) of Vermont Oxford Network (von) to the National Institute of Child Health and Development (nichd) Network. Pediatrics, 141: 508-513.
4. **Jobe A, Bancalari E (2001):** Bronchopulmonary dysplasia. Am J Respir Crit Care Med., 163: 1723–9.
5. **Galie N, Manes A, Branzi A (2004):** The endothelin system in pulmonary arterial hypertension. Cardiovascular Research, 61: 227-237.
6. **Li Y, Wang W, Zhang D (2019):** Maternal diabetes mellitus and risk of neonatal respiratory distress syndrome: a meta-analysis. Acta Diabetol., 56 (7): 729–740.
7. **Patrick D, Bo Yoon Ha, Mahesh R. (2021):** Hyaline Membrane Disease Imaging. https://emedicine.medscape.com/article/409409-overview
8. **Ballard J, Khourg J, Werdg K *et al.* (1991):** New Ballard score, expanded to include extremely premature infants. Journal of Pediatrics, 119: 417- 423.
9. **Tapia J, Agost D, Alegria A *et al.* (2006):** NEOCOSUR Collaborative Group11 Bronchopulmonary dysplasia: incidence, risk factors and resource utilization in a population of South American very low birth weight infants. J Pediatr (Rio J), 82 (1): 15-20.
10. **Ehrenkranz R, Walsh M, Vohr B (2005):** Validation of the National Institutes of Health consensus definition of bronchopulmonary dysplasia. Pediatrics, 116: 1353-1360.
11. **El Shemi M, Tawfik S, Khafagya S *et al.* (2016):** Endothelin 1 as a predictor marker for bronchopulmonary dysplasia in preterm neonates with respiratory distress syndrome. Journal of Neonatal-Perinatal Medicine, 10: 79–83.
12. **Dravet-Gounot P, Torchin H, Goffinet F *et al.* (2018):** Bronchopulmonary dysplasia in neonates born to mothers with preeclampsia: Impact of small for gestational age. PLoS One, 13 (9): e0204498.
13. **Demirel N, Bas A, Zenciroglu A (2009):** Bronchopulmonary Dysplasia in Very Low Birth Weight Infants. Indian J Pediatr., 76 (7): 695-698.
14. **Hansen A, Henriksen T, Wisborg K *et al.* (2007):** Risk of respiratory morbidity in term infants delivered by elective cesarean section: a cohort study. BMJ., 336: 85–7.
15. **Niu J, Munshi U, Siddiq M *et al.* (1998):** Early increase in endothelin-1 in tracheal aspirates of preterm infants: Correlation with bronchopulmonary dysplasia. The Journal of Pediatrics, 132 (6): 965-970.
16. **Fagan K, McMurtry I, Rodman D (2001):** Role of endothelin-1 in lung disease. Respir Res., 2: 90–101.
17. **Benzing J, Stabile O, Szinnai G *et al.* (2012):** Plasma Pro-Endothelin-1 and Respiratory Distress in Newborn Infants. J Pediatr., 160: 517-519.
18. **Kuo C, Chou Y, Lein R *et al.* (2001):** Study of plasma Endothelin-1 concentration in Taiwanese neonates with respiratory distress. Change Gung Medicine, 24: 239-244.
19. **Benjamin A, Silveira R, Procianoy R (2005):** Umbilical cord blood and neonatal endothelin-1 levels in preterm newborns with and without respiratory distress syndrome. Brazilian Journal of Medical and Biological Research, 38 (9): 1417-1422.
20. **Kojima T, Isozaki-Fukuda Y, Takedatsu M *et al.* (1992):** Plasma endothelin-1 like immunoreactivity levels in neonates. Eur J Pediatr., 151: 913-915.
21. **Charitharth V, Namasivayam A (2015)**: Biomarkers, Early Diagnosis, and Clinical Predictors of BPD. Clin Perinatol., 42 (4): 739-754.