Research Article

Plasma L-Arginine Levels in Preterm Newborns with Respiratory Distress Syndrome

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Abstract

Background: L-arginine is the precursor of nitric oxide which plays an important role on pulmonary circulation and pulmonary vascular tone. **Objective:** To estimate plasma L-arginine levels in preterm newborns with respiratory distress syndrome (RDS) and to correlate these levels with the severity of the disease. **Subjects and Methods:** This study included 56 preterm newborns divided into two groups: RDS group included 34 preterm newborns with RDS. The control group included 22 preterm newborns that did not develop RDS within the first 6 hours of life. Studied newborns were subjected to perinatal history, neonatal examination, assessment of Apgar score at 5 minutes, and respiratory system was carefully examined using the Downes´ score for grading of RDS. Laboratory work up included complete blood count, C-reactive protein, arterial blood gases, and estimation of L-arginine levels in plasma by ELISA. Plain chest X-ray was also done for cases of RDS. Follow up for 1 month to all cases were done to detect their outcome. **Results:** Plasma L-arginine levels were significantly lower in the RDS group than in the control group (p=0.000). In the RDS group: plasma levels of L-arginine were significantly higher in newborns whose mothers received antenatal steroids compared to those who did not receive (p=0.002). There were significant positive correlations between plasma L-arginine levels and Apgar score (r=0.534; p=0.001), blood pH (r=0.755; p=0.000), PaO_2(r=0.892; p=0.000), and with HCO_3 (r=0.843; p=0.000). On the other hand, there were significant negative correlations between plasma L-arginine levels and Downes´ score (r= -0.830; p=0.000), and with $PaCO_2$ (r= -0.737; p=0.000). No significant correlation was found between plasma L-arginine levels and gestational age or birth weight (r=0.276; p=0.114, and r=0.295; p=0.09 respectively). Plasma L-arginine levels were significantly lower in newborns who died or developed bronchopulmonary dysplasia than in those who were improved (p=0.036). The diagnostic value of plasma L-arginine levels in newborns with RDS at a cut-off < 54.25 μmol/ml had a sensitivity of 94.1%, a specificity of 95.5%, a positive predictive value of 97%, a negative predictive value of 91.3%, and an accuracy of 94.6% for prediction of neonatal RDS. **Conclusion:** Preterm newborns with RDS had lower plasma levels of L-arginine compared with non-RDS newborns. L-arginine is a good simple objective marker of RDS severity and prognosis. It is also a reliable marker for diagnosis of RDS. Lastly, its level does not need age or weight correction.

Key words: respiratory distress syndrome, L-arginine, preterm newborns.

Introduction

Neonatal respiratory distress syndrome (RDS), also known as hyaline membrane disease, is a major cause of death in the newborn, and affects approximately 1% of all live births.[1] It is also a common emergency that responsible for 30-40% of admissions in the neonatal period.[2] Neonatal RDS refers to respiratory compromise presenting at or shortly after birth specifically as a result of a deficiency of pulmonary surfactant; an endogenous detergent that serves to decrease the surface tension within the alveoli, thereby preventing alveolar collapse.[3] The RDS is a common lung disorder in premature infants as their lungs are not able to make enough surfactant. Without enough surfactant, the

lung collapse and the infants have to work hard to breathe, this cause lack of oxygen which can damage baby's brain and other organs.[4]

In addition to the immaturity of air sacs and surfactant deficiency, several studies suggested that there was increased pulmonary vascular resistance and poor ventilation-perfusion matching in preterm infants with severe RDS and respiratory failure. The mechanisms involved, however, were unknown.[5,6]

L-arginine is a known modulator of pulmonary vessels.[7] Arginine is an α-amino acid. The L-form is one of the 20 most common natural amino acids. In mammals, it is classified as a semi-essential or conditionally essential amino acid, depending on the developmental stage and health status of the individual.[8] Preterm infants are unable to synthesize or create arginine internally, making the amino acid nutritionally essential for them.[9]

L-arginine; the only known biologic substrate for nitric oxide (NO) formation, plays an important role on pulmonary circulation and pulmonary vascular tone.[10] The NO is synthesized from L-arginine by a family of NO synthase (NOS) isoforms.[11] The L-arginine-NO signaling pathway has emerged as one of the key second messenger systems involved in the regulation of normal blood pressure, vascular resistance, preservation of endothelial function, and protection against ischemic-reperfusion damage.[12]

The aim of this work was to estimate plasma L-arginine levels in preterm newborns with RDS and to correlate these levels with the severity of the disease.

Subjects and Methods

This study was a prospective comparative study included 56 preterm newborns. They were selected from the neonatal intensive care unit (NICU) of Children, Gynecology and Obstetrics Hospital of Minia University during the period from the first of November 2015 to the end of August 2016. They were divided into two groups:

1. RDS Group:

This group included 34 randomly selected preterm newborns with RDS. They were 17 (50%) males and 17(50%) females. Their gestational ages ranged from 30 to 36 weeks with a mean value of 32.38±1.6 weeks.

The diagnosis of RDS was confirmed by the presence of typical clinical and radiological signs of the disease in preterm infants.[13] all cases were followed up for 1 month and cases that did not improve and depended on oxygen therapy were considered as having bronchopulmonary dysplasia (BPD). In addition, newborns that died before 1 month while in the NICU due to respiratory failure from RDS were recorded. Oral consent was taken from each case caregiver before the study. The study was approved by the Ethical Committee Board of our Faculty.

Inclusion criteria:

1. Newborns with a gestational age of ≤ 36 weeks.
2. Newborns with a birth weight of ≤2.5 Kg.
3. Appearance of respiratory distress symptoms during the first 6 hours of life (tachypnea > 60/min, nasal flaring, subcostal and intercostal retractions, expiratory grunting, and cyanosis) and presence of typical radiological findings of RDS.

Exclusion criteria:

1. Full term newborns.
2. Premature newborns with 5-min Apgar score of less than 5.
3. Premature newborns with necrotizing enterocolitis (NEC), sepsis, pulmonary hypertension, and cardiac or other congenital malformations.
4. Mothers with a history of preeclampsia, diabetes mellitus and infections (e.g., chorioamnionitis).

2. Control Group:

The control group included 22 preterm newborns who did not develop RDS within the first 6 hours of life. They were matched to the diseased group as regards gestational age and sex. They were 13 (59.1%) males and 9(40.9%) females. Their gestational

ages ranged from 30 to 36 weeks with a mean value of 33±1.9 weeks. They presented no signs of fetal distress, and the Apgar score was > 7 at 1 and 5 minutes.

All newborns included in this study were subjected to:

Full antenatal, natal, and postnatal history. Assessment of Apgar score at 5 minutes. Thorough neonatal examination.

Gestational age was assessed by date of the last menstrual period, the ultrasonography, and confirmed by the method of expanded New Ballard Score.[14] Respiratory system was carefully examined using the Downes´ score[15] for grading of RDS. Plain chest X-ray for group I only (postero-anterior view). Laboratory work up included complete blood count (CBC), C-reactive protein (CRP), arterial blood gases, and estimation of L-arginine levels in plasma by enzyme-linked immunosorbent assay (ELISA) kit (Glory Science Co., Ltd, USA, catalog No 90119). Assay range:
2µmol/ml→600µmol/ml.

⊗ **Limitation:**

Our study could be improved if echocardiography was performed simultaneously with blood assays to measure pulmonary artery pressure. Unfortunately, this was unavailable in the NICU or the hospital where the study was done.

3. Specimen collection:

Arterial blood samples (3 ml) were obtained from all subjects under sterile aseptic techniques in the first 2 hours of life. We used the blood to analyze blood gases (0.5 ml), CBC (0.5 ml), and CRP (1ml). The rest of each sample (1 ml) was centrifuged for 15 minutes at 1000xg within 30 minutes of collection and plasma samples were stored at -20˚C until the time of L-arginine assay.

Statistical Methods

Analysis of the data was done using SPSS, version 20. The following statistical tests were used:

1. Mean and standard deviation (SD) to describe quantitative data.
2. Number and percent distribution to describe qualitative data.
3. Student t test was used to compare between two groups as regards quantitative data.
4. ANOVA test was used for comparison between more than two groups as regards quantitative data.
5. Chi-square test (X^2) was used to compare between two groups as regards qualitative data.
6. Pearson correlation (r) was used to correlate two quantitative variables.
7. Receiver Operating Characteristic (ROC)[16] analysis was used to evaluate the sensitivity, specificity, positive predictive value, negative predictive value, and accuracy of plasma L-arginine levels for prediction of neonatal RDS.

For all tests, a probability (p) of less than 0.05 was considered significant.

Results

A total of 56 preterm newborns were enrolled in the study. The study group consisted of 34 preterm newborns who developed RDS in the first 6 hours of life and the control group consisted of 22 preterm newborns who did not develop RDS. Regarding the follow up at the age of 1 month in newborns who developed RDS, our results revealed that: 25(73.5%) improved, 4(11.8%) developed BPD, and 5(14.7%) died. The results of the present study are illustrated in tables 1-7 and in figures 1& 2.

Table (1) shows that there were no statistically significant differences between RDS group and the control group regarding sex, gestational age, and birth weight (all p> 0.05). Newborns with RDS had significantly lower mean Apgar score and significantly higher mean Downes´ score than controls (p=0.006, and p=0.000 respectively). There were no statistically significant differences between RDS group and the control group regarding residence, maternal age, mode of delivery, and antenatal steroids administration (all p> 0.05).

Table (2) shows that the mean neonatal plasma L-arginine level was significantly lower in the preterm newborns who developed RDS than in the control group (p=0.000).

Table (3) shows that in the RDS group, the mean plasma level of L-arginine was significantly higher in newborns whose mothers received antenatal steroids compared to those who did not receive antenatal steroids (p = 0.002).

Table (4) shows that in the RDS group, the differences between plasma L-arginine levels when compared across the different stages of chest X-ray demonstrated a significant decrease with increased severity of chest X-ray findings (p= 0.003).

Table (5) shows that plasma L-arginine levels were significantly lower in newborns who died or developed BPD than in those who were improved at 1 month of age (p=0.036).

Table (6) shows that there was a significant positive moderate correlation between plasma L-arginine level and Apgar score (r=0.534; p=0.001), and significant positive strong correlations with blood pH (r=0.755; p=0.000), PaO_2 (r=0.892; p=0.000), and with HCO_3 (r=0.843; p=0.000). On the other hand, there was a significant negative strong correlation between plasma L-arginine level and Downes´ score (r= -0.830; p=0.000), and a significant negative moderate correlation with $PaCO_2$ (r= -0.737; p=0.000). Finally, there were insignificant positive fair correlations between plasma L-arginine level and both gestational age and birth weight (r=0.276; p=0.114, and r=0.295; p=0.09 respectively).

Table (7) shows that plasma L-arginine level as a diagnostic test at a cut-off < 54.25 µmol/ml had a sensitivity of 94.1%, a specificity of 95.5%, a positive predictive value of 97%, a negative predictive value of 91.3%, and an accuracy of 94.6% for prediction of neonatal RDS.

Table (1): Demographic and clinical characteristics of the studied groups

Variable	RDS Group (n= 34)	Control Group (n= 22)	Test value X^2 or t	p-value
Sex: n (%)				
Male	17 (50%)	13 (59.1%)	0.444	0.505
Female	17 (50%)	9 (40.9%)		
Gestational age (weeks):				
Range	30-36	30-36		
Mean ± SD	32.38±1.6	33 ±1.9	1.315	0.194
Birth weight (kg):				
Range	1.45-2.10	1.55-2.3		
Mean ± SD	1.71± 0.19	1.74± 0.1	0.681	0.499
Apgar score (5 min):				
Range	6-9	7-9		
Mean ± SD	7.29±0.87	7.9±0.6	2.87	0.006*
Downes´ score:				
Range	4-9	0-2		
Mean ± SD	5.91±1.29	1±0.5	17	0.000*
Residence: n (%)				
Urban	12(35.3%)	7(31.8%)	0.072	0.788
Rural	22(64.7%)	15(68.2%)		
Maternal age (years):				
Range	19.5-24	20-25.5		
Mean ± SD	21.9±1.02	22.3±1.1	1.389	0.170
Mode of delivery: n (%)				
Vaginal	10 (29.4%)	8 (36.4%)	0.296	0.586
Cesarean	24 (70.6%)	14 (63.6%)		
Antenatal steroids: n (%)				
Positive	18 (52.9%)	10 (45.5%)	4.851	0.584
Negative	16 (47.1%)	12 (54.5%)		

*p<0.05 is significant.

Table (2): Plasma L-arginine levels in the RDS group and the control group

Variable	RDS Group (n= 34)	Control Group (n= 22)	Test value -t	p-value
L-arginine level (µmol/ml)				
Range	14.4 - 55.4	53.2 - 97.1		
Mean ± SD	29.83 ±9.98	79.19 ±11.15	17.27	0.000*

*p<0.05 is significant.

Table (3): Plasma L-arginine levels in newborns in the RDS group regarding antenatal steroids administration

Variable	Newborns with antenatal steroids (n=18)	Newborns without antenatal steroids (n=16)	Test value - t	p-value
L-arginine levels (µmol/ml)				
Range	20.3-55.4	14.4-35.3		
Mean± SD	34.64±10.69	24.41±5.51	3.441	0.002*

*p<0.05 is significant.

Table (4): Plasma L-arginine levels in the RDS group regarding the chest X-ray findings

Variable	Stage 1 Reticulo-granular (n= 7)	Stage 2 mild air bronchogram (n= 13)	Stage 3 severe air bronchogram (n= 12)	Stage 4 white lung (n= 2)	Test value F	p-value
L-arginine level (µmol/ml):						
Range	21 - 55.4	21.5 - 43.3	14.4 - 40.4	18.2 -23.2		
Mean ± SD	40.43 ±13.31	29.89±5.94	25.09 ±7.17	20.7±3.54	5.92	0.003*

*p<0.05 is significant.

Table (5): Plasma L-arginine levels in relation to outcome in newborns with RDS

Variable	Improved (n=25)	Death or BPD (n=9)	Test value -t	p-value
L-arginine level (µmol/ml):				
Range	19.9-55.4	14.4-40.4		
Mean ± SD	31.96±10.02	23.91±7.5	2.19	0.036*

*p<0.05 is significant.

Table (6): Correlations between plasma L-arginine levels and different variables in the RDS group

Variable	plasma L-arginine level (µmol/ml)	
	r	p-value
Gestational age (weeks)	0.276	0.114
Birth weight (Kg)	0.295	0.09
Apgar score (5 min)	0.534	0.001*
Downes´ score	- 0.830	0.000*
Blood pH	0.755	0.000*
PaO_2 (mmHg)	0.892	0.000*
$PaCO_2$ (mmHg)	- 0.737	0.000*
HCO_3 (mEq/L)	0.843	0.000*

*p<0.05 is significant.

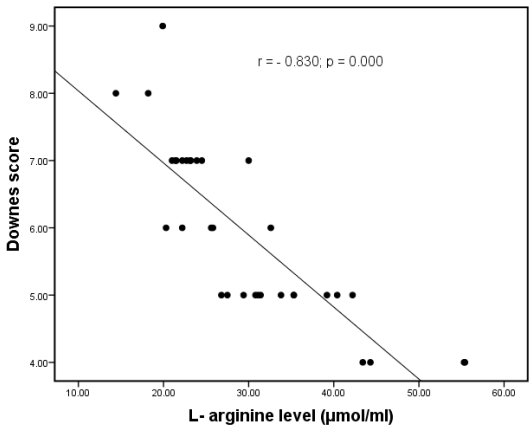

Figure (1): Correlation between plasma L-arginine levels and Downes score in preterm newborns with RDS

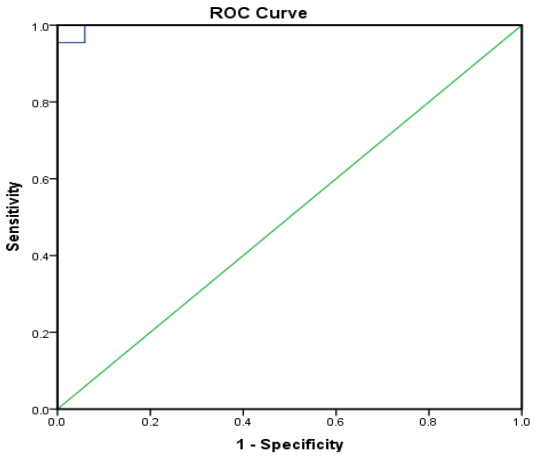

Figure (2): ROC curve for detection of the best cut off point of plasma L-arginine to predict RDS

Table (7): Diagnostic value of plasma L-arginine levels in newborns with RDS

	Cut-off	Sens (%)	Spec (%)	PPV (%)	NPV (%)	acc
L-arginine levels (µmol/ml)	< 54.25	94.1	95.5	97	91.3	94.6

Abbreviations: Sens, sensitivity; Spec, specificity; PPV, positive predictive value; NPV, negative predictive value; acc, accuracy.

Discussion

L-arginine is the precursor of NO which plays an important role on pulmonary circulation and pulmonary vascular tone. The aim of this work was to estimate plasma L-arginine levels in preterm newborns with RDS and to correlate these levels with the severity of the disease.

In the current study, the mean plasma L-arginine levels were significantly lower in preterm newborns who developed RDS than in the control group. This was in accordance with Canpolat et al.,[17] who found the same relationship between L-arginine level and the occurrence of RDS. Also our result agreed with the results of

El-Sayed et al.,[18] and Elfiky et al.,[19] who pointed out decrease serum L- arginine in preterm infants with RDS.

The relationship between low plasma L-arginine level and the occurrence of RDS could be explained by a "consumption" theory. Newborns with RDS might use more L-arginine for producing more NO to reduce pulmonary vascular tone via NO synthase than would patients without RDS.[17] This theory was also suggested by Vosatka et al.,[20] to be the mechanism that explains the coincident presence of low concentrations of NO metabolites and low plasma concentrations of L-arginine in newborns with persistent pulmonary hypertension. Pearson et al.,[21] confirmed this theory by his study that found that newborns with persistent pulmonary hypertension had significantly lower plasma concentrations of both L-arginine and NO metabolites.

Another possible mechanism involves patients' genetic variabilities and polymorphisms, related to enzymes with a role in L-arginine or NO production. This second theory was used in genetic polymorphism research where neonatal pulmonary hypertension patients were included, and NO synthase gene was investigated. However, there was no relationship between polymorphism of the NO synthase gene and neonatal pulmonary hypertension.[17] It was speculated that in stressed environment (i.e., after hypoxia), prolonged inhibition of NO synthetase activity produces activation of leukocytes, capillary leak, the release of secondary mediators, and subsequent regulation of vascular tone.[22]

In the current study, the mean plasma level of L-arginine was significantly higher in newborns whose mothers received antenatal steroids compared to those who did not receive antenatal steroids in the RDS group. This could be explained by that antenatal steroid administration enhanced surfactant synthesis which in turn decreased the severity of RDS which might decrease the L-arginine consumption.

Concerning different correlations, there was a negative strong correlation between plasma L-arginine level and the degree of respiratory distress as estimated by Downes´ score? This result was in accordance with Canpolat et al.,[17] who found that blood L-arginine concentration was inversely related to increasing severity of RDS. He used the oxygenation index [(Mean alveolar pressure X FiO_2X100)/ PaO_2] to determine the severity of the disease. This finding most likely reflects the consumption of L-arginine or could be explained by a generalized increase in catabolism in these infants. A similar finding was observed by Elfiky et al.[19] On the other hand, El-Sayed et al.,[18] in their study on 71 premature infants (gestational age 29-35 weeks) with and without RDS demonstrated no relation between blood L-arginine levels and the severity of RDS.

In the current study, we used Downes´ score to estimate the degree of severity of RDS. However, this score is a subjective method that depends on the skills of the medical personnel. On the other hand, the oxygenation index which was used by Canpolat et al.,[17] is an objective method that requires special equipments and endo-tracheal tube should be installed with its side effects.

The prognostic value of L-arginine estimation, proved by its correlation with Downes´ score in our study and oxygen-nation index in Canpolat et al.,[17] study, propose it as a better predictive test for RDS severity butting in mind being a simple objective laboratory test that doesn't require skilled personnel or special invasive equipments.

Our results showed that there were no relations between plasma L-arginine level and patients' gestational age or birth weight and this means that no correction is needed for the plasma L-arginine level as regard age or weight of the newborns.

Regarding the correlations of plasma L-arginine to other variables in the RDS group, there was a significant positive

moderate correlation between plasma L-arginine level and Apgar score, and significant positive strong correlations with blood pH, PaO_2, and with HCO_3. On the other hand, there was a significant negative moderate correlation between plasma L-arginine level and $PaCO_2$. The results of Elfiky et al.,[19] were in agreement with ours.

Regarding the outcome of newborns with RDS, plasma L-arginine levels were significantly lower in newborns who died or developed BPD than in those who were improved at 1 month of age. This result disagreed with the study of El-Sayed et al.,[18] who demonstrated no relation between blood L-arginine levels and the subsequent complications and pointed out that L-arginine was lower in infants with RDS but it did not differ between infants who died or developed BPD and those who did not.

Our statistical data analysis reached a cutoff point value for plasma L-arginine to predict RDS to be < 54.25 µmol/ml. Therefore, we could assume that newborns suspected to develop RDS should be sampled for plasma L-arginine. Those who will have levels above our cutoff value are at low risk for developing RDS. On the other hand, newborns with lower levels are at great risk to develop RDS and this risk increases with the more decrease in the L-arginine level.

The criticism to this assumption is the possibility of presence of other associated conditions or diseases that may affect plasma L-arginine level (e.g. NEC). This necessitates considering the clinical condition of the newborn that support a clinical diagnosis and exclude another, for example NEC is not manifested in the first few hours of life while RDS is commonly presented.[23] It also necessitates considering further investigational workup on this marker and its cutoff values in other diseases.

Conclusion

This study demonstrated that preterm newborns with RDS had lower plasma levels of L-arginine compared with non-RDS newborns. L-arginine is a good simple objective marker of RDS severity and prognosis. It is also a reliable marker for diagnosis of RDS. Lastly, its level does not need age or weight correction.

Recommendations:

1. Estimation of L-arginine level for newborns with suspected RDS to determine both occurrence and severity of RDS.
2. Further studies to know the effect of L-arginine supplementation for the newborns with RDS are recommended.
3. Further studies are indicated to know the effect of maternal administration of L-arginine to improve fetal and neo-natal outcome.
4. Further studies on this marker and its cutoff values in other neonatal diseases such as NEC.

References

1. Guyer B, Hoyert DL, Martin JA, Ventura SJ, MacDorman MF, Strobin DM (1999): Annual summary of vital statistics Pediatrics; 104:1229 - 46.
2. Mathai S, Raju C, Kanitkar M (2007): Management of Respiratory Distress in the Newborn. MJAFI; 63: 269-72.
3. Fantz CR, Powell C, Karon B, Parvin CA, Hankins K, Dayal M, Sadovsky Y, Johari V, Apple FS, Gronowski AM (2002): Assessment of the diagnostic accuracy of the TDx-FLM II to predict fetal lung maturity. Clin Chem.; 48(5): 761-65.
4. Pramanik AK (2012): Respiratory Distress Syndrome. Available at:http://emedicine.medscape.com/article/976034-overview.
5. Su PH and Chen JY (2008): Inhaled nitric oxide in the management of preterm infants with severe respiratory failure. J Perinatol.; 28(2):112-16.
6. Dani C and Pratesi S (2013): Nitric oxide for the treatment of preterm infants with respiratory distress syndrome. Expert Opin Pharmacother; 14(1):97-103.
7. Howell K., Costello C. M., Sands M., Dooley I., and McLoughlin P (2009): l-Arginine promotes angiogenesis in the chronically hypoxic lung: a novel mechanism ameliorating pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 296: 1042-50.

8. Tapiero H, Mathé G, Couvreur P, Tew K.D (2002): "L-Arginine". Biomedicine and Pharmacotherapy 56(9): 439-45.
9. Wu G, Jaeger A, Bazer W, and Rhoads M (2004). "Arginine deficiency in preterm infants: biochemical mechanisms and nutritional implications". *J* Nutr Biochem.; 15:442-51.
10. Hattenbach LO, Allers A, Klais C, Koch F, Hecker M (2000). L-Arginine-nitric oxide pathway-related metabolites in the aqueous humor of diabetic patients. Invest Ophthalmol Vis Sci.; 41(1):213-17.
11. Moncada S and Higgs A (1993): The L-arginine-nitric oxide pathway. N Engl J Med.; 329(27):2002-12.
12. Yang Z and Kaye DM (2006). Endothelial dysfunction and impaired L-arginine transport in hypertension and genetically predisposed normotensive subjects. Trends Cardiovasc Med.;16(4):118-24.
13. Miller MJ, Fanaroff AA, Martin RJ (2002): Respiratory disorders in preterm and term infants. In: Fanaroff AA, Martin RJ (eds). Neonatal-Perinatal Medicine Disease of the Fetus and Infant (7th ed) Vol. 2. St Louis: Mosby: 1025-49.
14. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R (1991): New Ballard score expanded to include extremely premature infants. J Pediatr;119:417-23.
15. Downes JJ, Vidyasagar D, Boggs TR Jr, Morrow GM III (1970): Respiratory distress syndrome of newborn infants. I. New clinical scoring system (RDS score) with acid—base and blood-gas correlations. Clin Pediatr (Phila); 9: 325-31.
16. Stephan C, Wesseling S, Schink T, et al., (2003): Comparison of eight computer programs for receiver-operating characteristic analysis. Clin Chem; 49:433- 39.
17. Canpolat FE, Yurdakok M, Yigit S, Korkmaz A, Tekinalp G (2005): Blood L-arginine levels in early respiratory distress syndrome. Pediatr Pulmonol; 40(6):511-14.
18. El-Sayed M, Sherif L, Said RN, El-Wakkad ASE, El-Refay A, Aly H (2011): Endothelin-1 and L-arginine in preterm infants with respiratory distress. Am J Perinatol; 28:129-36.
19. Elfiky A, Abdelmotaleb S, Ismail Y, Morshed E. (2012): Blood l-arginine level in early respiratory distress syndrome. Journal of Arab Child; 23(4): 269- 74.
20. Vosatka RJ, Kashyap S, Trifiletti RR (1994): Arginine deficiency accompanies persistent pulmonary hypertension of the newborn. Bioj Neonate; 66: 65-70.
21. Pearson DL, Dawling S, Walsh WF, Haines JL, Christman BW, Bazyk A, Scott N, Summar ML. Neonatal pulmonary hypertension--urea-cycle intermediates, nitric oxide production, and carbamoyl-phosphate synthetase function. N Engl J Med. 2001; 344: 1832-38.
22. Augusto LA, Li J, Synguelakis M, Johausson J, Chaby R (2002): Structural basis for interactions between lung surfactant protein C and bacterial lipopolysaccharide. J Biol Chem; 277(26):23484-92.
23. Gomella T, Cunningham M, Eyal F, and Tuttle D (2013): Respiratory management. In: Gomella Neonatology: Management, Procedures, On-call Problems, Disease, and Drugs. 7th ed Lange Clinical Science; 48-67.