The Egyptian Journal of Hospital Medicine (April 2012) Vol. 47 , Page 166– 175

Assessment of Cord Blood Vascular Endothelial Growth Factor Levels and Circulating $CD34^+$ Cells in Preterm Infants with Respiratory Distress Syndrome

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Abstract

Background:

Respiratory distress syndrome **(RDS)** secondary to surfactant deficiency is a common cause of mobility and mortality in premature infants. Vascular endothelial growth factor **(VEGF)** is a major angiogenic factor and prime regulator of endothelial cells proliferation. So, VEGF may contribute to surfactant secretion and pulmonary maturation. Additionally, circulating $CD34^+$ stem – progenitor cells are elevated along with its mobilizing cytokines in neonatal RDS. **Aim of work:** This study aimed to elucidate the role of cord blood VEGF and the circulating $CD34^+$ cells in preterm infants with and without RDS.

Patients & method:

This study was conducted on 55 preterm neonates divided into 25 preterm (15 males/ 10 females) without RDS with mean age of 31.60 ± 1.56 weeks and 30 preterm neonates with RDS (18 males/ 12 females) with mean age of 29.95 ± 1.09 weeks . Twenty healthy neonates (14 males/ 6 females) served as controls with mean age of 38.20 ± 3.57 weeks. All neonates were subjected to full history taking; thorough clinical examination and laboratory investigations including determination of VEGF levels in cord blood samples using ELISA and circulating $CD34^+$ cells in peripheral blood by flowcytometery.

Results:

The results of this study revealed that cord blood VEGF levels were significantly decreased in preterms with RDS versus preterms without RDS and controls with p values of both < 0.0001. Furthermore, the circulating $CD34^+$ cells were significantly increased in preterm infants with RDS versus preterm infants without RDS and controls ($p < 0.05$ & < 0.0001 respectively). Premature rupture of the membrane, gender of the newborn, birth weight and antenatal steroid administration had neither significant effect on the cord blood VEGF nor on the number of $CD34^+$ cells. There was inverse significant correlation between GA and the number of $CD34^+$ cells.

Conclusion:

It was concluded that low cord blood VEGF is associated with RDS and its level negatively correlated with the severity of the disease. Thus, it may play a role in recovery from acute lung injury in preterm infants. Moreover, the marked high level of circulating $CD34^+$ cells in preterms with RDS may give clear evidence of its promise therapeutic role in the future.
Key words: VEGF- $CD34^+$ - Respiratory distress syndrome

Introduction

Respiratory distress syndrome (RDS) previously known as hyaline membrane disease, is a common cause of morbidity and mortality in premature infants, the incidence is 56-60% in infants born between 27-28 weeks of gestation, and decreases with increasing gestational age (GA). **(Hornurubia and stark, 2004).** The development of RDS in premature infants is correlated with surfactant deficiency. **(Avery and Mead, 1995).** The outcome of RDS has improved in recent years with the increased use of antenatal steroids to improve pulmonary maturity, early postnatal surfactant therapy to replace surfactant deficiency, and gentle techniques of ventilation to minimize damage to the immature lung, these therapies also had resulted in the survival of preterm infants who are smaller and more ill **(Pramanik, 2002).**

Vascular endothelial growth factor (VEGF) is a specific mitogen for vascular cells and is a

Vascular endothelial growth factor (VEGF) is a specific mitogen for vascular cells and is a mediator of vascular permeability **(Ferrara *et al.*, 1992).** It is known to play a significant fetal and postnatal role in vascular development and participates in repair of lung injury in neonatal animals **(Pardanud *et al*., 2002).**

In lung from control infants VEGF is present in bronchial epithelial cells and in arterial medial smooth muscle cells and it is more intense in hypoplastic lung. **(Shehata *et al*.,1999).**

Previously, **Lassus *et al*., (2002)** demonstrated that infants with severe RDS had less vascular endothelial growth factor in their tracheal aspirate fluid during the early postnatal period than infants with milder RDS. They also mentioned that preterm infants with lower VEFG suffered prolonged and more severe RDS. These data suggested that VEGF might be a marker of pulmonary maturity.

Compernolle *et al*., (2002) demonstrated that intrauterine delivery or postnatal intratracheal instillation of VEGF stimulated conversion of glycogen to surfactant and protected preterm mice against RDS.

Hematopoietic stem and progenitor cells as assessed by $CD34^+$ expression, have been noted in the peripheral blood of human term neonates in levels comparable to those in umbilical cord blood (UCB). **(Li *et al*., 2001).** High levels of circulating $CD34^+$ cells in the blood of premature neonates would be associated with hastened recovery from lung injury **(Matthew *et al*., 2006).**

Previous data have suggested that circulating $CD34^+$ cells have the ability to differentiate into nonhematopoietic cells which may be involved in the tissue repair and may have a therapeutic role in a variety of disease such as bronchopulmonary dysplasia (BPD), a chronic lung disease that results in significant morbidity and mortality **(Zhang *et al*., 2008).** This study aimed to determine the level of circulating $CD34^+$ cells along with the cord blood concentration of VEGF in preterm infants with RDS during early postnatal life, and to determine whether they are associated with the disease severity and outcome or not.

Subjects and Methods

Fifty five preterm infants born at 25-34 weeks of gestation admitted to the neonatal intensive care unit (NICU) of Ain Shams University hospital were enrolled in this study (GA was estimated by last menstrual date or prenatal ultrasound).

Preterm neonates were be then followed up and then divided into 2 groups:

Group I- Preterm infants without RDS (n=25)
Group II- Preterm infants with RDS (n=30)

All the RDS infants received mechanical ventilation or nasal continuous positive airway pressure (nCPAP). Exogenous surfactant was administered within 2 hr after birth to infants with RDS who remained ventilator-dependent and who required a fraction of inspired O2 (FiO2) of more than 0.4 to maintain pulse oximeter saturation (SpO2) >90%. The signs of respiratory distress must develop through the first 4 hours and persist beyond 24 hours of age (Rudolph & Smith, 1960).

Full term infants (n = 20) without diffuse lung diseases admitted to the neonatal ward during the same period served as controls.

Infants were excluded from the study if there was evidence of prenatal maternal infection, any infection within the first 3 days of life, major congenital anomalies, hemolytic jaundice, or blood transfusion which might influence the number of $CD34^+$ cells**.**

All neonates will be subjected to:

- Patient information, including demographic characteristics.
- Perinatal, natal and family history, complications, medications taken by the mothers perinatally and mode of delivery.
- Duration of assisted ventilation and oxygen support, and length of hospital stay.
- Complete clinical, physical and neurological examination.
- APGAR score for neonates were obtained from medical records.
- Chest x rays and assessment for respiratory status.
- According to **Clementes *et al*.(1972)** respiratory distress was classified into 4 grades.

Laboratory investigations:

Cord blood was collected in heparinized syringes upon delivery and centrifuged within

15 minutes of collection. Plasma was kept at -70ºC until analysis.

Assay of plasma vascular endothelial growth factor: The level of VEGF was assayed by standardized enzyme-linked immunosorbent assay (ELISA, R&D Systems) in duplicate, according to the protocol recommended by the manufacturer **(Rodriguez *et al.*, 1998).**

Flow cytometry for measuring numbers of CD 34^+ cell/ µl:

Peripheral blood (1 ml) was collected in a tube containing heparin within 72 h after birth. About 0.1 ml blood was used for cytometric analysis. The expression of cell surface antigen $CD34^+$ was analyzed by the gating strategy of a modified ISHAGE protocol **(Barnett *et al.*, 1999).** 50 µl of peripheral blood was incubated with 10 µl of PE-conjugated anti-human CD34 and 10 µl of FITC-conjugated anti-human CD45 MAb (BD Biosciences, San Jose, CA, USA) at room temperature for 20 min. Anti-isotype antibody served as a control. Subsequently, red cells were lysed, the remainders were washed and finally resuspended in 400 µl phosphate-buffered saline. Flow cytometry was performed using a FACSCalibur flow cytometer (BD Biosciences, Mountain View, CA, USA). In total, 70,000 events were acquired. Circulating $CD34^+$ cells were expressed as absolute number and the percentage of total nucleated cells in peripheral blood.

Statistical analysis:

Results were expressed as mean± standard deviation or medians and range for continuous variables (APGAR scores), or as number and percentage for categorical variables. For comparison between two variables, the student's t-test was applied. ANOVA test served for analyses between concentrations of VEGF and $CD34^+$ in different RDS grades in preterms. $P<0.05$ was considered as statistically significant. **Pearson and spearman's** correlation test were used to correlate each parameter with different variants in the same group to differentiate between positive and negative correlations and to find significant difference **(Daniel, 1991).**

Results

The results were demonstrated through the following tables and figures:

The mean gestational age in full terms was 38.20±3.57weeks, and mean birth weight was 2816.00±261.30 gm. However, in preterms, gestational age was ranged between 25-34 weeks. Birth weights of the infants with RDS were 1780.25 ±168.93. They showed statistically significant decrease as compared to full term infants and preterms without RDS. Twenty four infants in this study were delivered by CS. Thirty one neonates received antenatal steroid treatment. Table 1 concluded that 30 neonates suffered from RDS, had lower gestational age, lower birth weights, a higher incidence of endotracheal tube intubation, a longer duration of intubation, and 10 of them needed surfactant therapy .

Table (1): Characteristics of preterms infants with or without RDS and full term infants

	Full term infants (Controls) (n=20)	Preterm infants without RDS (n=25)	Preterm infants with RDS (n=30)
Sex (M/F)	14/6	15/10	18/12
Gestational age (weeks) Mean±SD p-values	38.20±3.57	31.90±1.56 <0.001*	29.95±1.09 <0.001* NS**
Birth weights (gm) Mean±SD p-values	2816.00±261.30	1975.25±224.94 <0.0001*	1780.25±168.93 <0.0001* <0.0001**
Mode of delivery Vaginal ,n(%) CS ,n(%)	 17 (85) 3 (15)	 15 (60) 10 (40)	 16 (53.3) 14(46.7)
PROM, n(%)	0 (0)	7 (28)	8 (26.7)
Prenatal steroid, n(%)	0 (0)	14 (56)	17 (56.7)
Endotracheal intubation, n(%)	0 (0)	4 (16)	18 (60)
Duration of intubation (days)	0	2	14
Surfactant therapy,n(%)	0 (0)	0 (0)	10 (33.3)

*: Compared to Full term infants **: Compared to Preterm infants without RDS

P<0.001, considered highly significant , P<0.0001, considered very highly significant

PROM: Premature rupture of membrane

As seen in table (2) preterm infants with RDS had very high significantly lower cord blood VEGF level than those without RDS and Full term infants ($p<0.0001$). However, the numbers of $CD34^+$ in RDS infants had a significantly higher number than preterm controls without RDS (Mean, Range: 45.05 (10-115) vs 24.55 (2-99) cells/ µl; $p<0.05$) and than full term infants ($p<0.0001$).

Table (2): Levels of serum VEGF and CD34+ cells in preterms and full term infants

	Full term infants (Controls) (n=20)	Preterm infants without RDS (n=25)	Preterm infants with RDS (n=30)
Plasma VEGF (pg/ml) Mean±SD p-values	48.08±6.53	46.61±10.21 NS*	17.85±3.30 <0.0001* <0.0001**
CD34+ (cells/µl) mean±SD p-values	12.90±5.48	24.55±19.09 <0.05*	45.05±30.92 <0.0001* <0.05**

*: Compared to Full term infants **: Compared to Preterm infants without RDS
NS: non significant (P>0.05) P<0.05, considered significant
P<0.0001, considered very highly significant

Lower APGAR score was observed in preterm infants with RDS at 1 minute (Median: 3) and 5 minute (Median: 7) compared to preterm infant without RDS and full term infants. 40% of preterm infants with RDS was grade 1, 30% grade II, 20% grade III and 10% was grade IV (table 3).

Table (3): APGAR score and RDS grades in the three studied groups

	Full term infants (Controls) (n=20)	Preterm infants without RDS (n=25)	Preterm infants with RDS (n=30)
APGAR score(median:range) 1 min 5 min	 7 (5-9) 9 (7-10)	 6 (1-9) 8 (4-10)	 3 (0-7) 7 (3-9)
RDS grade (%) I II III IV	 0 0 0 0	 0 0 0 0	 40% 30% 20% 10%

Levels of VEGF were very significantly lower in preterms infants with RDS than preterms without RDS. The level of VEGF was decreased significantly with the grades of RDS ($P<0.0001$). However, levels of $CD34^+$ were significantly higher in preterms with RDS than preterms without RDS. The levels of $CD34^+$were increased significantly with the grades of RDS ($P< 0.0001$) (table 4).

Table (4): Comparison of VEGF and $CD34^+$ in different RDS grades in preterm infants with RDS

	Preterm infant with RDS (n=30)		
		VEGF	$CD34^+$cells
RDS grade(%) I II III IV	 40 30 20 10	 37.05±5.07 28.20±5.09 17.36±7.18 12.21±8.19	 28.95±13.65 45.00±29.09 48.30±24.20 51.25±29.02
p-values		<0.0001*	<0.0001*

*: Compared RDS grades with each other using ANOVA test

Table (5) shows no significant differences between positive and negative maternal history of PROM, newborns delivered by different modes (vaginal or CS), and newborns whose mothers received steroids antenatally or not was observed.

Table (5): Comparison of VEGF and CD34+ in different epidemiological data and steroid administration in both preterms infants

	Preterm infant without RDS (n=25)		Preterm infant with RDS (n=30)	
	VEGF	$CD34^+$ cells	VEGF	$CD34^+$ cells
PROM				
Positive	40.25±8.21	25.50±16.00	12.95±4.90	45.05±32.02
negative	45.61±10.31	20.12±18.09	18.85±2.30	40.95±30.00
p-values	NS	NS	NS	NS
Mode of delivery				
Vaginal	46.90±9.29	22.90±19.01	17.31±3.45	42.31±27.12
CS	42.00±11.25	26.55±14.11	13.85±4.30	46.05±24.30
p-values	NS	NS	NS	NS
Steroid administration				
Positive	49.63±10.41	26.40±14.26	19.24±5.15	45.90±30.02
negative	47.01±9.21	29.55±16.18	15.80±3.99	49.55±26.91
p-values	NS	NS	NS	NS

NS: non significant (P>0.05)

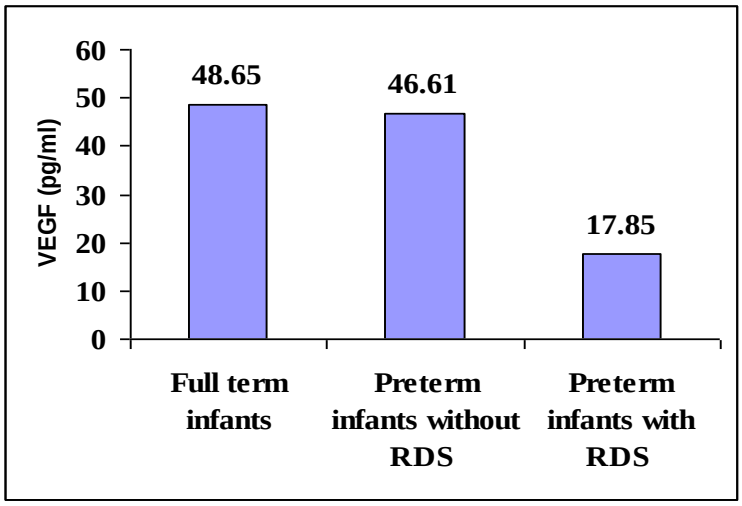
No significant correlations were observed between VEGF and both of gestational age and birth weights in both preterm infants. However, the number of $CD34^+$ cells show significant inverse correlations with gestational age but not with birth weights in all preterm infants. Also, no significant correlation was observed between blood cord VEGF and number of $CD34^+$ cells in all preterm infants with or without RDS (table 6).

Table (6): Correlations of VEGF and $CD34^+$ with both of Gestational age and Birth weights in preterm infants

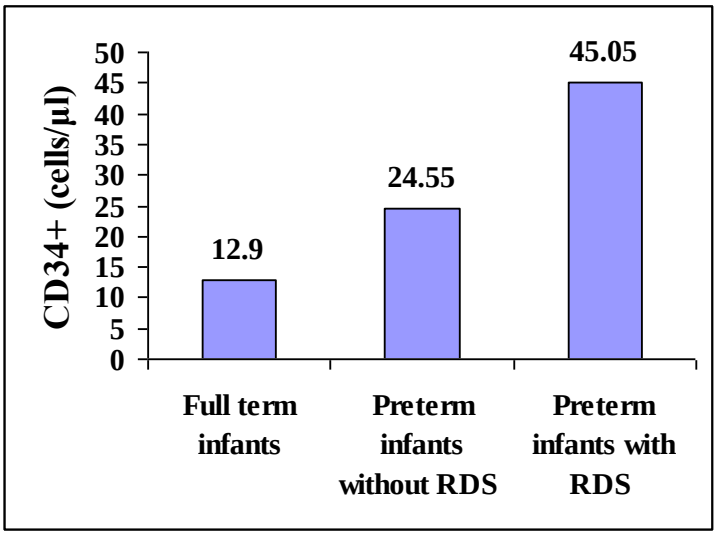
	Preterm infant without RDS (n=25)		Preterm infant with RDS (n=30)	
	VEGF	$CD34^+$ cells	VEGF	$CD34^+$ cells
Gestational age				
r	0.1145	-0.5651	0.2019	-0.4320
p-value	NS	<0.05	NS	<0.05
Birth weights				
r	0.0958	0.1390	0.0119	0.1108
p-value	NS	NS	NS	NS
$CD34^+$ cells				
r	0.1216		0.0988	
p-value	NS		NS	

NS: non significant (P>0.05) P<0.05, considered significant

Fig (1,2) show levels of VEGF and numbers of $CD34^+$ cells in preterms with or without RDS compared to full term infants.



Fig(1): Levels of plasma VEGF in full terms infants and preterms with and without RDS



Fig(2): Numbers of $CD34^+$ cells in full terms infants and preterms with and without RDS

Discussion

Preterm delivery and development of RDS continue to be one of the main cause of neonatal morbidity and mortality, despite exhaustive efforts the rate of prematurity and development of RDS had not decreased, however neonatal survival rates have increased **(Lewis *et al*., 1996).**

The incidence of RDS is inversely proportionate to gestational age (GA) and affects about 50% of infants born at or less than 28 weeks of gestation, while the greatest risk factor is prematurity, maternal and fetal infection and asphexia (Pramanik, 2002). Increase evidence suggests that VEGF may contribute to surfactant secretion and pulmonary maturation **(Po-Nien *et al*., 2005).**

The present study demonstrated that cord blood VEGF levels are significantly lower in preterm infants with clinically diagnosed RDS with mean gestational age of 29.95 ± 1.09 weeks and with body weight with a mean of 1780.25 ± 168.93 gm than preterm infants without respiratory distress, with mean gestational age of 31.90 ± 1.56 weeks and body weight with a mean of 1975.25 ± 224.94 gm and full term control. These results came in agreement of Compernolle et al. (2002) who showed that VEGF can regulate fetal lung maturation and suggested that the pneumotrophic effect of VEGF may have therapeutic potential for lung maturation in preterm infants, in addition.

Lassus *et al*., (2002) concluded that VEGF levels in tracheal aspirate fluid was lower in infants with severe RDS, and the correlation existed between VEGF and the functional

maturity of alveolar type II cells indicated that VEGF contribute to lung maturation and surfactant production.

Infants with RDS may develop acute lung injury as bronchopulmonary dysplasia (BPD) and they had low cord blood VEGF levels not due to their lower gestational age but because infants who eventually developed BPD required higher inspiratory oxygen concentrations which has been reported to decrease VEGF expression by alveolar epithelial cells. The present study proved the same previous result since low cord blood VEGF levels were found in preterm infants with RDS especially those who needed high oxygen concentration.

The present study demonstrated no significant effect on the level of cord blood VEGF as regarding premature rupture of the membrane, use of antenatal steroid and mode of delivery **Abdel Hady *et al*., (2007)** confirmed our result as they demonstrated that the cord blood VEGF levels in preterm infants with RDS not affected by the sex of the new born mode of delivery (although CS is a risk factor of developing RDS) maternal disease and PROM. However **Lassus *et al*., (2002)** reported that higher levels of VEGF in tracheal fluid aspirate from preterm infants born to mothers suffering from chorioamnionitis.

Pio-Nien *et al*., (2005) also concluded that antenatal steroid treatment was not associated with changes in cord blood VEGF levels **Tsao *et al*., (2005)** reported that no correlation between antenatal steroid administration and cord blood VEGF levels, they also reported that pulmonary VEGF levels increased with low dose antenatal dexamethasone administration and suppressed with high dose of dexamethasone.

The present study demonstrated that the cord blood VEGF levels significantly decreased in infants with severe RDS . These data indicated that VEGF levels contribute to lung maturation and surfactant synthesis **(Compernolle *et al*., 2002).**

Hassan *et al*., (2009) recorded that at birth levels of serum VEGF in infants who developed RDS and BPD were lower than those with no BPD at birth and remained lower, although not significantly until 3 weeks of age, so this finding at birth can be used as biological predictor for the development of BPD.

Abdel Hady *et al*., (2007) also postulated that cord blood VEGF level was significantly lower in preterm infants with RDS as compared to preterm infants without RDS and controls. They also found that infants with sever RDS especially those with small gestational age, low birth weight and low APGAR score at 1 and 5 minutes had significantly lower cord blood VEGF levels than those with mild RDS.

Our results indicated no correlation between cord blood VEGF levels and both GA and BW. The same results were obtained by **Lassus *et al*., (2002)** and **Pio-Nien *et al*., (2005)** who reported that infants with severe RDS had lower tracheal aspirate concentration of VEGF with no correlations between it and birth weight or gestational age.

A small number of $CD34^+$ cells normally circulate in peripheral blood; they directly reflect hematopoiesis and also believed to be involved in tissue repair **(Gupta *et al*., 2007).** A previous study showed that extremely preterm neonates with RDS had high levels of CD34+ cells, and also they reported that the use of umbilical blood obtained from this population could increase the hematopoietic stem and progenitor cells(HSCP) as assessed by $CD34^+$, yield thereby improving the potential for clinical applications **(Bizzaro *et al*., 2007).**

Our data recorded that number of $CD34^+$ cells are higher in preterm infants with severe RDS than preterm controls and full term healthy infants. The mean CD^{34+} stem cells counts in preterm RDS infants were significantly higher than those obtained from the peripheral blood of adults (2 cells/mL) this discrepancy is likely related to the prematurity of the patients populations **(Li *et al*., 2001).** It's possible that fluctuation and subsequent discrepancies in the levels of the circulating $CD34^+$ cells in each individual may coincide with the timing of transfer of hematopoiesis from liver to bone marrow, which varies from neonates to neonates.

In the present study, a significant inverse correlation between CD34+ cells and gestational age was observed. In accordance to this finding, **Yuanyuan *et al*., (2010)** observed that the number of $CD34^+$ cells was inversely

related to the age at sampling . Moreover,the percentage of CD^{34+} cells was significantly higher in control infants with GA < 32 weeks than those > 32 weeks (P < 0.01).

This study revealed that the circulating $CD34^{+}$ cells levels increased in preterm infants with RDS than preterm infants without RDS and controls, **Bizzarro *et al*., (2007)** explained this results on the fact that GA differed in their study. **Yuanyuan *et al*., (2010)** reported that preterm infants with RDS had increased levels of circulating stem and progenitor cells in the early postnatal life which are mobilized into peripheral circulation early in post natal life.

No correlation was found between VEGF levels concentration and the number of $CD34^{+}$ cells in this study, which may related to the potential inadequacy of the study. Meanwhile, **Baker *et al*., (2009)** didn't find any relationship between circulating CD^{34+} cells and plasma level of VEGF in premature neonates.

We concluded that low cord blood VEGF is associated with RDS and its level negatively correlated with the severity of the disease and the duration of ventilation.Thus, it may play a role in recovery from acute lung injury in preterm infants. Moreover, the marked high level of circulating $CD34^{+}$ cells in preterms with RDS may give clear evidence of its promise therapeutic role in the future.

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قياس مستويات عامل النمو الوعائي البطاني فى دم الحبل السرى والخلايا الجزعية +CD34 في الأوعية الدموية الطرفية عند حديثي الولادة المبتسرين المصابين بمتلازمة صعوبة التنفس

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تعتبر الإصابة بمتلازمة صعوبة التنفس المعروف سابقًا بمرض الغشاء الزجاجي من الأسباب الشائعة لإصابة حديثي الولادة ناقصي النمو باضطراب التنفس وكذلك ارتفاع معدلات الوفيات بينهم.
ولهذا فإن حدوث متلازمة صعوبة التنفس في الأطفال ناقصي النمو مرتبط بنقص عامل السرفاكتنت. إدخال عامل السرفاكتنت البديل أظهر قدرة واضحة على تقليل ومنع حدوث مضاعفات صعوبة التنفس في حديثي الولادة. وقد أوضحت كثير من الدراسات السابقة أن معامل النمو الوعائي البطاني له دور كبير في نمو وتكاثر الخلايا المبطنة للأوعية الدموية لكلاً من الجنين والأم أثناء الحمل وقد أشارت الدراسات أيضًا أن الخلايا الجزعية المتمثلة في $CD34^+$ لها دور كبير في علاج ونمو خلايا الرئة في الأجنة.
وتهدف هذه الدراسة إلى عرض وتوضيح دور معامل النمو البطاني الرحمي في دم الحبل السري وكذلك الخلايا الجزعية $CD34^+$ في الأوعية الدموية الطرفية لحديثي الولادة ناقصي النمو سواء كانوا مصابين بمتلازمة صعوبة التنفس أم لا.
وقد أجريت هذه الدراسة على 55 طفلاً ناقصي النمو يتراوح عمرهم الرحمي ما بين 25-34 أسبوع وكذلك على 20 طفلاً كاملي النمو وغير مصابون بمتلازمة صعوبة التنفس ويتراوح عمرهم الرحمي ما بين 36-41 أسبوع كعينة ضابطة وقد تم اختيار الاطفال من وحدة الرعاية المركزة للأطفال المبتسرين بجامعة عين شمس.
ولقد تم استبعاد أي رضع مولودين لأمهات مصابات بالعدوى قبل الولادة وكذلك تم استبعاد أي رضع أصيبوا بالعدوى البكتيرية في أول ثلاثة أيام من الولادة.
وقد تم تقسيم الأطفال ناقصي النمو المشاركين في البحث إلى مجموعتين:
المجموعة الأولى: تشتمل على 25 طفلاً حديث الولادة 15 ذكر، 10 أنثى غير مصابون بمتلازمة صعوبة التنفس ويتراوح عمرهم الرحمي ما بين (31.9 ± 1.56) أسبوع ومتوسط أوزانهم (1975.25 ± 224.94 جرام)

المجموعة الثانية وتشتمل على 30 طفلاً حديثى الولادة (18 ذكر ،12 أنثى) مصابون بمتلازمة صعوبة التنفس ويتراوح عمرهم الرحمي بين (29.95 ± 1.09 أسبوعًا) ومتوسط أوزانهم (1780.25 ± 168.93 جرامًا).

المجموعة الضابطة: تشتمل الأطفال كاملي النمو وعددهم 20 كعينة ضابطة ومتوسط عمرهم الرحمي 38.2 ± 3.57 أسبوعًا ومتوسط أوزانهم 2816 ± 261.3 جرامًا.

وقد خضع جميع المشاركون في البحث إلى:
1- دراسة التاريخ المرضى (تاريخ ما قبل الولادة – تاريخ الولادة – التاريخ لعائلي – طريقة الولادة).
2- تحديد معيارأبجر.
3- فحص أكلينكي وعصبي كامل.
4- أشعة سينية على الصدر.

اختبارات معملية وتشتمل على:
أخذ عينة من دم الحبل السري لقياس معامل النمو الوعائي البطاني بواسطة جهاز الإليزا.
أخذ عينة من الأوعية الدموية الطرفية لقياس عدد الخلايا الجزعية ($CD34^+$) بواسطة جهاز الفلوسيتوميتر.

النتائج:
وجد نقص ذو دلالة إحصائية في مستوى عامل النمو البطاني في دم الحبل السري في الأطفال ناقصي النمو المصابين بمتلازمة صعوبة التنفس عنه في الأطفال ناقصي النمو الذين لم يصابوا بالمرض والعينة الضابطة.
ما وجد أن عدد الخلايا ($CD34^+$) في الأوعية الدموية الطرفية للأطفال المصابين بمتلازمة صعوبة التنفس تزيد زيادة كبيرة عنها في الأطفال ناقصي النمو الغير مصابون بالمرض وكذلك بالنسبة للعينة الضابطة.
الأطفال المصابون بمتلازمة صعوبة التنفس عند الولادة غالبًا ما يكونوا ناقصي النمو – وعمرهم الرحمي صغير وكذلك يعانون من انخفاض معيار أبجر ويحتاجون إلى أوكسجين أو أجهزة تنفسي خارجية لمساعدتهم في التنفسي.
ولقد لوحظ أن مستوى معامل النمو البطاني الوعائي ينقص نقصًا شديدًا كلما زادت شدة الإصابة بمتلازمة صعوبة التنفس عند الولادة وكذلك يرتفع عدد خلايا $CD34^+$ في الدم لنفس السبب.
وقد أثبتت الدراسة أن عدد خلايا $CD34^+$ في الأوعية الدموية الطرفية تتناسب تناسبًا عكسيًا مع العمر الرحمي لحديثي الولادة في حين أن مستوى معامل النمو البطاني الوعائي في الحبل السري لا يتأثر بها.
كما أثبتت الدراسة أيضاً أن علاج الأم بالكورتيزون قبل الولادة وطريقة الولادة وكذلك نوع الجنين تعتبر كلها عوامل غير مؤثرة تماما على مستويات كل من معامل النمو البطاني الوعائي في الحبل السري وعدد خلايا $CD34^+$ في الأوعية الدموية الطرفية للمبتسرين.
وقد خلص البحث إلى أن زيادة مستوى معامل النمو الوعائي البطاني في دم الأطفال حديثي الولادة يمكن استخدامه كمؤشرلكفاءة الرئتين للتأكد من عدم إصابة الأطفال بمتلازمة صعوبة التنفس . كما أن عدد الخلايا الجزعية المتمثلة في $CD34^+$ يمكن الاستفادة منها في معالجة إصابات الرئة عند الأطفال حديثي الولادة المبتسرين.