Egyptian Journal of Medical Microbiology Volume 25 / No.4 / October 2016 17-24

ORIGINAL ARTICLE

Molecular Detection of *Ureaplasma urealyticum* and *Ureaplasma parvum* Colonization in Preterm Infants with Respiratory Distress Syndrome

[1]Randa Mohamed Kamal Abd-EL Raouf, [2]Safaa Shafik Imam, [3]Maha Muhammad Fathy, [3]Noha Nagi Salah El-Deen, [4]Amany Moussa Husseiny*

[1]Pediatrics Medical Studies Department, Institute of Postgraduate Childhood Studies, Ain Shams University
[2]Pediatrics Department, Faculty of Medicine, Ain Shams University
[3]Medical Microbiology & Immunology Department, Faculty of Medicine, Ain Shams University
[4]Pediatrics Department, El Matarriah Teaching Hospital

Key words:

Ureaplasma urealyticum, Ureaplasma parvum, RDS, PCR, Urease gene subunits

ABSTRACT

Background: *Early neonatal acquisition of Ureaplasma urealyticum (U. urealyticum) and Ureaplasma parvum (U. parvum), in the 1st 24 hours after birth and during passage of an infected maternal birth canal, have been linked to the development of respiratory diseases in premature newborns as a result of respiratory tract colonization. However, the role of these pathogens in the development of respiratory distress syndrome (RDS) still needs more clarification.* ***Objectives:*** *In this study we aimed to search for U. urealyticum and U. parvum in tracheal aspirates of premature infants with RDS by species-specific PCR assays and to evaluate the short term outcome of preterm infants with positive results.* ***Methodology:*** *Tracheal aspirate fluid samples from 35 preterm neonates with RDS with a mean gestational age of (32.76±2.48) weeks were collected within the first 24 hours after birth and analyzed for detection of U. urealyticum and U. parvum by species-specific PCR assays targeting urease gene subunits and adjoining spacer regions.* ***Results:*** *Ureaplasma spp. was detected in the tracheal aspirate of 6 (17%) preterm infants with RDS. Among these 6 positive cases; 5 (83%) were positive for U. parvum while only 1 case (17%) was positive for U. urealyticum. The preterm infants with positive results for Ureaplasma spp. had statistically significant higher degree of RD depending on the chest X ray grading results and longer durations of ventilation and hospitalization.* ***Conclusions:*** *Limited by the relatively small sample size, this work can be considered as a pilot study indicating a possible association between the severity of RDS in preterm infants and respiratory tract (RT) colonization with Ureaplasma spp. particularly U.parvum.*

INTRODUCTION

Respiratory distress syndrome (RDS) is a main cause of morbidity and mortality in the early neonatal period. Related to the degree of prematurity, it occurs in 7%-50% of neonates. It is also responsible for 30% - 40% of newborns' hospital admission [1]. Many studies confirmed an inverse relationship between RDS and gestational age [2, 3]. Despite the improvement in perinatal care, RDS still remains a major neonatal problem [4, 5].

According to previous studies in recent years, infection with *Ureaplasma* species (spp.) was found to be responsible for the increased liability of preterm neonates to RDS [6].

Corresponding Author:
Noha Nagi Salah El-Deen:
Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt
Email: dr.noha_nagi@hotmail.com; Tel.: +201093399611

These pathogens are readily transmitted venereally and vertically, either *in utero* or at delivery of the neonate [7]. *Ureaplasma parvum* (*U. parvum*) is generally the commonest *Ureaplasma* spp. detected in various clinical specimens; however, *Ureaplasma urealyticum* (*U. urealyticum*) is apparently more pathogenic in conditions such as male urethritis [8].

A number of studies attempted to relate *U. urealyticum* colonization to the development of respiratory diseases in high risk newborns (gestational age <28 weeks), but the effective role of *U. urealyticum* and *U. parvum* in the development of RDS when associated with prematurity remains controversial [9].

Aim of the work:

This study aimed to search for a possible association between the presence of *Ureaplasma* species (spp.) in the respiratory tract of pre-term infants and the development of RDS and to identify the most prevalent associated *Ureaplasma* spp. using species-specific PCR

assays. Moreover, the study aimed to evaluate the short term outcome of RDS in preterm infants with positive results.

METHODOLOGY

This cross sectional study included 35 premature neonates from Neonatal Intensive Care Unit (NICU) of El Matarriah Teaching Hospital within a time period of 6 months. The study protocol was approved by the ethical committee of the Institute of Postgraduate Childhood Studies and that of El Matarriah Teaching Hospital, and verbal consent was obtained from each parent of neonates included in the study. The study included 18 males and 17 females with a gestational age ranging from 28 to 36 wks. All of them were preterm (<37weeks) fulfilling the criteria of RDS (tachypnea, intercostal retractions, expiratory grunting, and a persistent oxygen requirement for more than 24 hours (F_iO_2 >0.4), and/or radiographic evidence of hyaline membrane disease)[10], and the need for assisted ventilation (mechanical ventilation either by tracheal intubation or by nasal continuous positive air ways pressure (CPAP), using the fractional inspired oxygen (F_iO_2>0.21), as the amount of oxygen delivered to the patient. Preterm newborns with major congenital abnormalities, intrauterine infection, CNS anomalies, CVS anomalies, chest anomalies, sepsis and babies who received surfactant were excluded from the study. Neonates born to mothers with history of chorioamnionitis, premature rupture of membranes (PROM) >18 hours or mothers who received antibiotics before delivery were also excluded.

All the studied neonates were subjected to detailed perinatal medical history taking including; (1) perinatal history (gestational age according to last menstrual period, data of maternal risk factors as PROM or use of antibiotics during pregnancy, chorioamnionitis, hypertension, and antenatal steroids administration), (2) natal history (mode of delivery, perinatal asphyxia and meconium aspiration) and (3) postnatal history **(**Apgar score at 1, 5 minutes after delivery and sex)

Thorough clinical examination was performed. It included; (1) general examination (heart rate, respiratory rate and core temperature, blood pressure, color, attitude, and determination of birth weight), (2) systemic examination (with laying stress on respiratory system as regards clinical grading of respiratory distress, and search for any signs of sepsis), (3) gestational age assessment by physical and neuromuscular evaluation using New Ballard score [11], (4) clinical assessment of respiratory distress using Silverman-Anderson scoring [12], and (5) the need for assisted ventilation (mechanical ventilation either by tracheal intubation or by nasal CPAP, using the fractional inspired oxygen (F_iO_2>0.21), as the amount of oxygen delivered to the patient

All included neonates were subjected to routine laboratory investigations (Complete blood picture, C reactive protein (CRP) and arterial blood gases), radiological investigations (a frontal chest radiograph was performed at birth and radiological severity of RDS was graded in 4 categories) [13].

Tracheal aspirates were collected from all included neonates for detection of *U. urealyticum and U. parvum* deoxy-ribo nucleic acid (DNA) by species specific PCR assays targeting urease gene subunits and adjoining spacer regions.

Sample Collection and PCR assays:

On 1st day after delivery, 1ml of normal saline was injected into the endotracheal tube of each neonate, and then tracheal aspirate was withdrawn into a sterile tube. The samples were transferred to the laboratory and stored at -20 ^oC till further processing and testing. DNA extraction for purification of genomic DNA was done using QIAgen DNA/RNA Kit® (QIAgen, USA). The PCR technique was performed with a final volume of 50 µl reaction mix; 25 µl of a 2x QIAgen HotStar Taq® Master Mix, 11 µl of RNase-free water, 2 µl of each primer (table 1), and 10 µl of tested DNA. Negative control (QIAgen® RNase-free water) and positive controls (provided by Prof. Ali Zaki) were included in each PCR run. The species-specific primer pairs used in our study (TIB MOBIOL, GmbH, Berlin, Germany) proved to distinguish the two species [14] (table 1).

The thermal cycler (thermo PxE 0.21, England), was programmed with the following cycling profile: QIAgen *Taq* DNA polymerase activation was performed by incubation at 95°C for 15 min, followed by 35 cycles of: 94°C for 45 s, 55°C for 45 s, and 72°C for 1 min with final extension at 72°C for 10 minutes. Identification of amplified products by gel electrophoresis according to Voytas [15] was done.

Table (1): Primers *for U. urealyticum and U. parvum* targeting urease gene subunits and adjoining spacer regions

Primer	*Sequence*	*Band*
UP 1	5' CAg gAT CAT CAA gTC AAT TTA g 3'	421bp
UP 2	5' AAC ATA ATg TTC CCC TTT TTA TC 3'	
UU1	5' CAg gAT CAT CAA ATC AAT TCA C 3'	419bp
UU2	5' CAT AAT gTT CCC CTT CgT CTA 3'	

Statistical analysis:

Data was tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 16. Descriptive statistics were calculated for the data in the form of Mean and Standard deviation (±SD). In the statistical comparison between the different groups, the significance of difference was tested using one of the following tests: Student's *t*-test, Chi-Square test (X^2-value), and Pearson correlation coefficient. *P* value was used as determinant as significance (P>0.05 was considered insignificant, P<0.05 was considered statistically significant (S), and P<0.001 was considered highly significant).

RESULTS

The demographic data, the clinical findings and the results of routine laboratory investigations of the studied premature neonates are presented in table (2).

PCR assays results revealed that six cases (17.1%) were positive for *Ureaplasma* spp. and 29 cases (82.9%) were negative. Five of the positive cases, were positive for *U. parvum* (14.3% of the whole study group) and one case was positive for *U.urealyticum* (2.9% of the whole study group) (Figure 1). Figure 2 shows the agarose gel electrophoresis of PCR final amplification products of some processed tracheal samples.

Table (2): Demographic data and the clinical and routine laboratory findings of the studied premature neonates

Characteristics	***No.= 35***
Sex	
Female	17 (48.5%)
Male	18 (51.5%)
Mode of delivery	
Vaginal	14 (40%)
Caesarian Section	21 (60%)
Gestational age (weeks)	32.76 ± 2.48
Weight (kg)	1.8 ± 0.5
Apgar score 1	3 [2-6]
Apgar score 5	6 [4-9]
PH	7.27 ± 0.11
CO_2 cmH_2O	40.73 ± 10.1
HCO_3 mmHg	20.59 ± 3.78
TLC(10^3/ccm)	13.98 ± 5.81
HB(g/dl)	14.88 ± 1.9
Plat. (10^3/ccm)	189.4 ± 49.3

Data are presented as mean ± standard deviation or median [interquartile range] for continuous variables and as number (percentage) for categorical variables

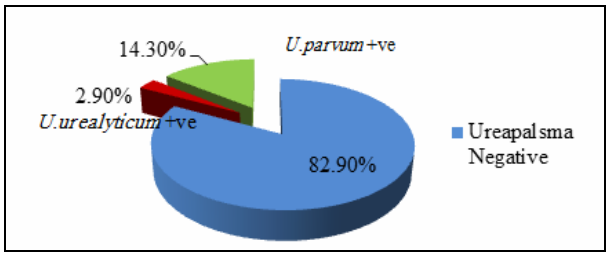

Fig. 1: Ureaplasma species-specific PCR assays results of tracheal aspirates of the studies preterm infants (n=35)

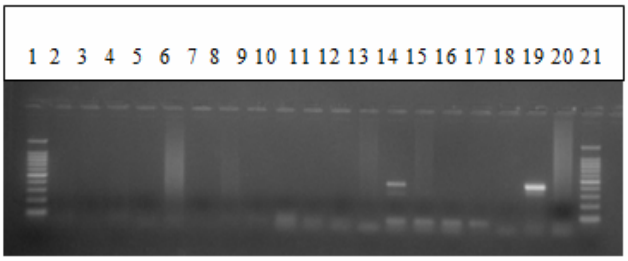

Fig. 2: Agarose gel electrophoresis of PCR final amplification products. Lanes 1, 21: Molecular size marker (ladder), Lane 19: positive control, Lane 20: negative control, Lane 14: positive sample (*Ureaplasma parvum*), rest of lanes show negative samples.

Comparing *Ureaplasma* spp. positive and negative groups, no statistically significant difference was found as regards sex, gestational age, birth weight, Apgar score, and mode of delivery. Also no statistically significant difference was found when comparing the results of blood gases testing and CBC (Table 3).

However, the preterm infants giving positive results in PCR assays for *Ureaplasma* spp. had statistically significant higher degree of RD (P= 0.01) depending on the chest X ray grading results (Table 4).

The results also showed that *Ureaplasma* spp. positive preterm infants with RD had statistically significant longer durations of ventilation and hospitalization (Table 5).

There were statistically significant negative correlations between chest x-ray scoring of RD with gestational age (r= -0.96, P= 0.002), and birth weight (r= -0.94, P= 0.006). Although positive correlations were found with duration of ventilation (r= -0.34, P= 0.51) and fate (r= -0.16, P= 0.77) yet, they were not statistically significant (P<0.05).

In the study's patients as a whole, 85.7% of the neonates survived while 14.3% died. It is worth noting that 3 of the 6 (50%) of *Ureaplasma* spp. positive infants with RD died while among the negative group only 6.9% (2/29) died and 93.1% survived.

Table (3): Demographic and clinical characteristics of patients with *Ureaplasma* spp. positive and negative PCR results

	Ureaplasma spp.		$t (x^{2*})$	P
	Positive No.=6	Negative No.= 29		
Age	32.33 ± 2.422	32.76 ± 2.488	0.4	0.7
Sex				
Male	2 (33.3%)	16 (55.2%)	0.9*	0.4
Female	4 (66.7%)	13 (44.8%)		
Weight	1.825 ± 0.5880	1.809 ± 0.4973	0.1	0.9
Apgar 1	3.33 ± 0.816	3.79 ± 1.346	1.1	0.3
Apgar 5	5.67 ± 1.366	6.14 ± 1.642	0.7	0.5
Mode of delivery				
Vaginal	3 (50%)	11 (37.9 %)	0.6*	0.7
Caesarian section	3 (50%)	18 (62.1 %)		
CBC				
TLC (10^3/ccm)	10.37 ± 5.760	15.57 ± 5.709	2.02	0.08
HB (gm/dl)	14.583 ± 2.9816	14.941± 1.6584	0.3	0.8
Plat. (10^3/ccm)	201.17 ± 54.521	193.34 ± 48.213	0.4	0.7
Blood gases				
PH	7.258 ± 0.1462	7.272 ± 0.1023	0.3	0.8
CO_2 cmH_2O	47.32 ± 14.327	39.37 ± 8.660	1.8	0.07
HCO_3 mmHg	21.85 ± 2.148	20.33 ± 4.019	0.9	0.4

Data are presented as mean ± standard deviation for continuous variables and as number (percentage) for categorical variables

t (x^{2*}): Student's *t*-test (Chi-Square test)

***p*: *P* value**

Table (4): Comparison between *Ureaplasma* spp. positive and negative groups as regards clinical and radiological degree of RD

		Ureaplasma spp.				*Total*		X^2	P
		Negative		Positive					
		No.	%	No.	%	No.	%		
RD Degree 1		25	86.2%	2	33.3%	27	77.1%	6.8	0.03
RD Degree 2		4	13.8%	4	66.7%	8	22.9%		
X-ray RDS grades	**1**	12	41.4%	1	16.7%	13	37.1%	10.8	0.01
	2	10	34.5%	1	16.7%	11	31.4%		
	3	6	20.7%	1	16.7%	7	20.0%		
	4	1	3.5%	3	50.0%	4	11.4%		

X^2: Chi-Square test

***p*: *P* value**

Table (5): Comparison between *Ureaplasma* spp. positive and negative groups as regards duration of ventilation, and duration of hospitalization

Variable	*Ureaplasma*	*No.=35*	*Mean*	*Std. Deviation*	t	P
Duration of hospitalization(days)	Negative	29	11.83	3.9	3.2	**0.003**
	Positive	6	17.9	5.6		
Duration of ventilation(days)	Negative	29	8.3	3.2	3.98	**0.0004**
	Positive	6	14.5	4.5		

t: Student's *t*-test

***p*: *P* value**

DISCUSSION

In our study we relied on the PCR assay for detection of *Ureaplasma* spp., directly from tracheal aspirate samples of the premature infants. PCR is more sensitive than culture for detection (<100 genome copies) of nonviable as well as viable *Ureaplasma* spp.[16]. Also use of PCR assay to detect Ureaplasma in different clinical samples has been shown to be comparable or superior to that of culture according to multiple studies [17, 18, 19, 20, 21]. Targeting the urease gene in the PCR enabled us to discriminate between the two *Ureaplasma* species as described in other studies [22, 23]. Use of PCR to detect Ureaplasma was supported by the results of a previous study which determined that patients with a positive PCR for *Ureaplasma* spp. but a negative amniotic fluid culture had a higher rate of significant neonatal morbidity than those with a negative culture and negative PCR($P<0.05$) [24]. The samples were collected within 24 hours after birth to avoid hospital acquired colonization or infection.

According to our results, *Ureaplasma* spp. were detected in tracheal aspirates of 17% pre-term infants with RDS (6 cases out of 35). Among these 6 cases: 5 (83%) were *U. parvum* and 1 (17%) case was *U. urealyticum* positive. Similar to our results, records from the Diagnostic Mycoplasma Laboratory at the University of Alabama at Birmingham show that *Ureaplasma* spp. were detected in 18% sequential endotracheal aspirates from preterm neonates with respiratory distress [25]. Other studies found that rate of respiratory tract colonization with *Ureaplasma* spp. in very low birth weight infants (<1,501g) ranges from 20%to 45%[16]. The difference in detection rates will reflect the frequency of maternal colonization in the lower urogenital tract of women in the population studied. Also many studies reported that detection rates vary inversely with gestational age[26,27,28,29,30]. In our study mean birthweight of the included preterm infants was 1800 g ± 500 and it was nearly the same in both *Ureaplasma* spp. positive and negative groups (≈1,800 g). Also the gestational age of the preterm infants in the included preterm infants was 32.76 ± 2.48 with no significant difference between the positive and negative groups. These findings may explain the relatively lower rate of detection of *Ureaplasma* spp. in our study. In support of this, Kafetzis et al. [28] demonstrated a vertical transmission rate of 60% for infants with a birth weight of <1,000 g versus only 15.3% for infants with birth weights of 1,500 g. These investigators also found that the overall Ureaplasma colonization rate was 10% for full-term infants versus 24% of preterm infants.

Our study confirmed previous observations of others[7,31] who found that *U. parvum* was the predominant *Ureaplasma* spp. colonizing the respiratory tract of preterm infants. In contrast, Heggie et al.[32] reported a similar distribution of the two species by species-specific PCR of culture-positive broths from endotracheal aspirates. Differences between the study results may be due to differences in study populations and different in epidemiological colonization pattern.

The studied neonates were 51.4% males and 48.5% females with male to female ratio of 1.04: 1. Some studies reported that boys are more likely to develop RDS than girls and more likely to die from the disease [33, 34, 35]. However, other researchers found that there was no statistical significant difference between male and female as regards occurrence of RDS and the rate of *Ureaplasma* spp. colonization [36, 37, 38].

The comparison between *Ureaplasma* spp. positive and negative groups was hindered by the low number of positive cases (6 cases) however, the results of the study showed that half of the *Ureaplasma* spp. positive tracheal samples collected from pre-term infants delivered vaginally and the other half from infants delivered by C.S. Similarly Patterson and colleagues, [39] documented that the mode of delivery had no effect on *U. urealyticum* isolation from respiratory tract of premature neonates and they suggested that lung inflammation might be initiated prenatally by *U. urealyticum* colonization of amniotic fluid or membrane. Different studies reported that *Ureaplasma* spp. can be transmitted from infected females to the fetus or neonate by at least three different routes including an ascending intrauterine infection in which the organisms gain access to the amniotic sac where they multiply and are then passed into the fetal lung even when fetal membranes are intact. Also fetal acquisition of *Ureaplasma* spp. can occur through a hematogenous route through placental infection. Finally, acquisition of these organisms by the neonate can occur through passage of an infected maternal birth canal with resultant colonization of the skin, mucosal membranes and respiratory tract [37, 40, 41, 42, 43, 44].

History of premature rupture of membranes was recorded in four out of the six (66.7 %) *Ureaplasma* spp. positive patients. Similarly, a study done by Pandey et al.[45] reported that infants colonized with *Ureaplasma spp.* are more likely to have been born following premature rupture of membranes than those not colonized.

The results of the present study documented that premature neonates with PCR positive tracheal aspirate for *Ureaplasma* spp. had a statistically significant higher RD chest x-ray scoring (P= 0.01), longer duration of ventilation with a mean of 14.5±4.5 days (P= 0.0004) and more days of hospitalization with a mean of 17.9±5.6 days (P= 0.003) when compared to those with *Ureaplasma* spp. negative aspirates. Other investigators [46] found similar results in 22 infants from whom *Ureaplasma* spp. were detected in blood as they experienced significantly more hospital stays and remained hospitalized for more days during the first 12 months of postnatal life than 18 infants without

infection. *In vitro* and *in vivo* studies demonstrated that *Urealasma* spp. may cause lung injury through a number of mechanisms including the inhibition of pulmonary surfactant by phospholipase A2 and the production of pro-inflammatory cytokines [47, 48, 49, 50]. Pro-inflammatory cytokines are believed to play role in mediating pathology through innate and adaptive immune responses. These include interleukin-1ß (IL-1ß), tumor necrosis factor alpha (TNF-), and IL-6. IL-1 ß and TNF- activate the immune system, produce inflammation, and induce the release of IL-6, which affects the proliferation of antibody-producing B cells but also limits pulmonary inflammation associated with pneumonia and hypoxia. In the mature immune system, activation of the inflammatory pathway is opposed by the production of cytokines such as IL-10 which down-regulate inflammation and host defense mechanisms in order to protect from an excessively strong response to stimuli. Small amounts of IL-10 in lung lavages of intubated preterm infants with respiratory distress suggests that the immature immune system has a limited ability to down-regulate the inflammatory response [49].

In contrast to our finding, other investigators found decreased incidence of RDS in infants < 28 weeks gestation who are colonized with *Ureaplasma spp*. However, many of these colonized infants progressed to develop chronic lung disease [36, 51]. The difference in the results could be attributed to many variables, including sampling frequency, the method of detection and population characteristics. Also the type of sample as it has been proposed that positive tracheal aspirates represent lower respiratory tract colonization and positive nasopharyngeal-samples represent upper respiratory tract colonization [39].

In this study 50% of *Ureaplasma* spp. positive cases died versus 6.9 % in *Ureaplasma* spp. negative cases. ***Arit et al.*** [52] demonstrated a high mortality rate in neonates with low gestational age and low birth weight**.** ***Duško et al.*** [53] also detected higher risk of mortality in infants with lower birth weight and shorter gestational age in 126 premature infants hospitalized at Pediatric Intensive Care Unit.

Several studies assessed the colonization of the lower respiratory tract by *Ureaplasma* spp. and particularly by *U. parvum* in preterm newborns and related it to RDS, but our study is the first to highlight the problem in Cairo, Egypt and paving the way for future studies to determine the relationship of *Ureaplasma* spp. virulence factors, host immune factors affecting pathogen susceptibility, and inflammatory variability, and interactions with environmental factors such as oxygen exposure.

Limitations of the study:

The study limited by the relatively small sized sample that was restricted to preterm infants with RDS in the 1st 24 hours. Wider scale studies are needed to allow evaluating the long term outcome of *Ureaplasma* spp. colonized preterm infants with RDS.

CONCLUSIONS

Colonization of the lower respiratory tract by *Ureaplasma* spp., particularly *U. parvum*, in preterm neonates increase the duration of ventilation and hospitalization in preterm infants with RDS, thus increasing hospital costs and exposing the colonized preterm infants to severe comorbidities and dangerous outcomes. The routine use of PCR could be useful to screen candidate babies for etiologic therapy in order to decrease the complications of RDS and to give a chance for catch up normal growth and development.

Acknowledgments*:*

We are grateful for Prof. Ali Zaki, Prof. of Medical Microbiology and Immunology, Ain Shams Faculty of Medicine who supplied us with the positive control for PCR assays.

REFERENCES

1. Varvarigou AA, Thomas I, Rodi M, Economou L, Mantagos S, Mouzaki A. RDS in Premature Infants Is Underscored by the Magnitude of Th1 Cytokine Polarization. Cytokine 2015; 58: 355-60.
2. Niknafs P, Faghani A, Afjeh A, Moradinazer M, Bahman-Bijari B. Management of Neonatal RDS Employing Acute Care of at-Risk Newborns Respiratory Sequence Protocol (ACoRN) versus Early Nasal Continuous Positive Airway Pressure Protocol. Iran J Pediatr 2014; 24: 57-63.
3. Jing L, Hai Ying C, Hua-Wei W, Xiang YK. The Role of Lung Ultrasound in Diagnosis of RDS in Newborn Infants. Iran J Pediatr 2015; 25: e323.
4. Gortner L, Tutdibi E. Respiratory Disorders in Preterm and term Neonates: An Update on Diagnostics and Therapy. Z Geburtshilfe Neonatol 2011; 15: 145-151.
5. Saboute M, Kashaki M, Bordbar A, Khalessi N, Farahani Z. The Incidence of Respiratory Distress Syndrome among Preterm Infants Admitted to Neonatal Intensive Care Unit: A Retrospective Study. Open Journal of Pediatrics 2015; 5(4): 285-289
6. Sung TJ, Xiao L, Duffy L, et al. Frequency of ureaplasma serovars in respiratory secretions of preterm infants at risk for BPD. Pediatr Infect Dis J 2011; 30: 379–83
7. Waites KB, Xiao L, and Paralanov V, et al. Molecular methods for the detection of Mycoplasma and Ureaplasma infections in humans: a paper from the 2011 William Beaumont Hospital Symposium on molecular pathology. J Mol Diagn 2012; 14: 437–50.

8. Xiao L, Paralanov V, Glass JI. Extensive horizontal gene transfer in ureaplasmas from humans questions the utility of serotyping for diagnostic purposes. J Clin Microbiol 2011; 49(8): 2818–2826.
9. Kasper D, Mechtler T, Böhm J, et al. In utero exposure to Ureaplasma spp. is associated with increased rate of bronchopulmonary dysplasia and intraventricular hemorrhage in preterm infants. Journal of Perinatal Medicine 2011; 39(3): 331–336.
10. Welty S, Hansen TN, Corbet A. Respiratory distress in the preterm infant in: Taeusch HW, Ballard RA, Gleason GA (Eds), Avery, s diseases of the newborn, 8th edn, El Sevier, Saunders: pp. 2005; 687-703.
11. Ballard JL, Khoury JC, Wedig K, et al. New Ballard score, expanded to include extremely premature infants. The Journal of Pediatrics 1991; 119(3):417–423.
12. Silverman WA, Anderson DH. Silverman score: A system for grading severity of underlying lung disease. Pediatr 1956; 17:1
13. Fletcher BD. The radiology of RDS and its sequelae. In Stern, L. (ed.), HMD: Pathogenesis and Pathophysiology. Orlando: Grune and Stratton 1984; 119–143.
14. Kong F, Ma Z, James G, Gordon S, Gilbert G. Species Identification and Subtyping of Ureaplasma parvum and Ureaplasma urealyticum Using PCR-Based Assays. J Clin Microbiol 2000; 38(3): 1175–1179
15. Voytas D. Gel electrophoresis. Curr Protoc Immunol 2001; 10: unit 10.4
16. Viscardi RM. Ureaplasma species: Role in Neonatal Morbidities and Outcomes. Arch Dis Child Fetal Neonatal Ed 2014; 99(1):F87-F92.
17. Abele-Horn M C, Dressel WP, Zimmermann A, Vahlensieck W, Pfaff F, Ruckdeschel G. Polymerase chain reaction versus culture for detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* in the urogenital tract of adults and the respiratory tract of newborns. Eur J Clin Microbiol Infect Dis 1996; 15:595–598.
18. Blanchard AJ, Duffy HL, Baldus K, Cassell GH. Detection of *Ureaplasma urealyticum* by polymerase chain reaction in the urogenital tract of adults, in amniotic fluid, and in the respiratory tract of newborns. Clin Infect Dis 1993; 17(Suppl. 1):S148–153.
19. Colaizy TT, Kuforiji T, Sklar RS, Pillers AM. PCR methods in clinical investigations of human Ureaplasmas: a minireview. Mol Genet Metab 2003; 80:389–397.
20. Luki NP, Boucher LM, Doray B, Turgeon J, Brousseau R. Comparison of polymerase chain reaction assay with culture for detection of genital mycoplasmas in perinatal infections. Eur J Clin Microbiol Infect Dis 1998; 17:255–263.
21. Yoon BH, Romero R, Kim M, Kim EC, Kim T, Park JS, et al. Clinical implications of detection of *Ureaplasma urealyticum* in the amniotic cavity with the polymerase chain reaction. Am J Obstet Gynecol 2000; 183:1130–1137.
22. Cao X, Wang Y, Hu X, Qing H, Wang H. Real-time TaqMan PCR assays for quantitative detection and differentiation of *Ureaplasm urealyticum* and *Ureaplasma parvum*. Diagn Microbiol Infect Dis 2007; 57: 373–378.
23. Yi J, Yoon BH, Kim EC. Detection and biovar discrimination of *U. urealyticum* by real-time PCR. Mol Cell Probes.2005 ;19:255–260
24. Yoon BH, Romero R, Lim JH, Shim SS, Hong JS, Shim JY, et al. The clinical significance of detecting *Ureaplasma urealyticum* by the polymerase chain reaction in the amniotic fluid of patients with preterm labor. Am J Obstet Gynecol 2003; 189:919–924.
25. Bowman ED, Dharmalingam A, Fan WQ, Brown F, Garland SM. Impact of erythromycin on respiratory colonization of *Ureaplasma urealyticum* and the development of chronic lung disease in extremely low birth weight infants. Pediatr Infect Dis J 1998; 17:615–620.
26. Chua KB, Ngeow YF, Lim CT, Ng KB, Chye JK. Colonization and transmission of *Ureaplasma urealyticum* and *Mycoplasma hominis* from mothers to full and preterm babies by normal vaginal delivery. Med J Malaysia 1999; 54:242–246.
27. Dinsmoor MJ, Ramamurthy RS, Gibbs RS. Transmission of genital mycoplasmas from mother to neonate in women with prolonged membrane rupture. Pediatr Infect Dis J1989; 8:483–487.
28. Kafetzis DA, Skevaki CL, Skouteri V, Gavrili S, Peppa K, Kostalos C, et al. Maternal genital colonization with Ureaplasma urealyticum promotes preterm delivery: association of the respiratory colonization of premature infants with chronic lung disease and increased mortality. Clin Infect Dis 2004; 39:1113–1122.
29. Sanchez PJ, Regan JAX. Vertical transmission of *Ureaplasma urealyticum* from mothers to preterm infants. Pediatr Infect Dis J 1990; 9:398– 401.
30. Syrogiannopoulos GA, Kapatais-Zoumbos K, Decavalas GO, Markantes CG, Katsarou VA, Beratis NG. *Ureaplasma urealyticum* colonization of full term infants: perinatal acquisition and persistence during early infancy. Pediatr Infect Dis J 1990; 9:236–240.
31. Katz B, Patel P, Duffy L, Schelonka RL, Dimmitt RA, Waites KB. Characterization of Ureaplasma isolated from preterm infants with and without BPD. J Clin Microbiol 2005; 43:4852-4.

32. Heggie AD, Bar-Shain D, Boxerbaum B, Fanaroff AA, O'Riordan MA, Robertson JA. Identification and quantification of Ureaplasmas colonizing the respiratory tract and assessment of their role in the development of CLD in preterm infants. Pediatr Infect Dis J 2001; 20: 854–859
33. Maly MV, Hallman M, Gluck L. The hormonal regulation of lung maturation. Clin Prenatal 2000; 6: 377
34. Pramanik AK. RDS. Am J Respir Crit Care Med 2000; 165(9): 1322-8
35. Mathur N, Pankaj G, Mishra T. Predictors of fatality in neonates requiring mechanical ventilation. Indian predictors 2005; 42:645-650.
36. Hannaford K, David AT, Jeffery H, John E, Blyth K, Gilbert G. Role of *U.urealyticum* in lung disease of prematurity. Arch Dis Child Fetal Neonatal Ed 1999; 81:F162–F167.
37. Cassell GH, Waites KB, Crouse DT, Rudd PT, Canupp KC, Stagno S, et al. Association of *U. urealyticum* infection of the lower respiratory tract with CLD and death in VLBW infants. Lancet1988; 30(2): 240-244.
38. Panero A, Pacifico L, Rossi N, Roggini M, Chiesa C. *Ureaplasma urealyticum* as a cause of pneumonia in preterm infants: analysis of the white cell response. Arch Dis Child Fetal Neonatal Ed 1995; 73:F37-40.
39. Patterson AM, Taciak V, Lovchik J, Fox RE, Campbell AB, Viscardi RM. *U.urealyticum* respiratory tract colonization is associated with an increase in IL1-β and TNF-α relative to IL-6 in tracheal aspirate of preterm infants. Pediatr Infect Dis J 1998; 17:321-8.
40. Cassell GH, Davis RO, Waites KB, Brown MB, Marriott PA, Stagno S, et al. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16–20 weeks of gestation: potential effect on outcome of pregnancy. Sex Transm Dis 1983; 10:294–302.
41. Izraeli S, Samra Z, Sirota L, Merlob P, Davidson S. Genital mycoplasmas in preterm infants: prevalence and clinical significance. Eur J Pediatr1991; 150:804–807.
42. Kelly VN, Garland SM, Gilbert GL. Isolation of genital mycoplasmas from the blood of neonates and women with pelvic infection using conventional SPS-free blood culture media. Pathology 1987; 19:277–280.
43. Ollikainen J, Hiekkaniemi H, Korppi M, Sarkkinen H, Heinonen K. *Ureaplasma urealyticum* infection associated with acute respiratory insufficiency and death in premature infants. J Pediatr 1993; 122:756–760.
44. Waites KB, Crouse DT, Cassell GH. Systemic neonatal infection due to Ureaplasma urealyticum. Clin Infect Dis 1993; 17(Suppl. 1): S131–S135.
45. Pandey A, Dhawan B, Gupta V, Chaudhry R, Deorari AK. Clinical significance of airways colonization with Ureaplasma urealyticum in premature (<34 wk) neonates. Indian J Med Res 2007; 125(5):679-84.
46. Ollikainen J. Perinatal *Ureaplasma urealyticum* infection increases the need for hospital treatment during the first year of life in preterm infants. Pediatr Pulmonol 2000; 30:402–405.
47. Kotecha S, Hodge R, Schaber JA, Miralles R, Silverman M, Grant WD. Pulmonary *Ureaplasma urealyticum* is associated with the development of acute lung inflammation and chronic lung disease in preterm infants. Pediatr Res 2004; 9 55:61-68.
48. Schrama AJ, De Beaufort AJ, Jansen SM, Sukul YM, Poorthuis BJ, Berger HM. Phospholipase A2 is present in mechonium and inhibits the activity of pulmonary surfactant: an in vitro study [abstract]. Pediatr Res 2000; 47:375A.
49. Manimtim WM, Hasday JD, Hester L, Fairchild KD, Lovchik JC, Viscardi RM. *Ureaplasma urealyticum* modulates endotoxininduced cytokine release by human monocytes derived from preterm and term newborns and adults. Infect Immun 2001; 69:3906-3915.
50. Cultrera R, Lemercier B, Seraceni S, De Carlo A, Rawadi G, Contini C. Human monocyte cytokine mRNA expression and production after stimulation with membrane lipopeptides of different Mycoplasma species. Clin Microbiol Infect 1999; 5(Suppl 3):247.
51. Watterberg KL, Demers LM, Scott SM, Murphy S. Chorioamnionitis and early lung inflammation in infants in whom BPD develops. Pediatrics 1997; 99 (1): 144.
52. Arit P, Nighat H, Zubair AK, Abdul Sattar S. Frequency, Causes and Outcome of Neonates with Respiratory Distress Admitted to Neonatal Intensive Care Unit, National Institute of Child Health, Karachi. Journal Pakistan Medical Association 2015; 65, 771-775.
53. Duško F, Vladislav M, Aleksandar S, Antoni H, Aspasija S, Ljiljana K, Mica K. Mortality risk factors in premature infants with respiratory distress syndrome treated by mechanical ventilation. Srpski Arhiv za Celokupno Lekarstvo 2005;133(1-2): 29-35