INTRODUCTION

Inflammation plays a central role in respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD) in the preterm infant. Although antenatally increased concentrations of cytokines in the blood or amniotic fluid stimulate lung maturation and prevent the postnatal development of RDS, they may also initiate the development of BPD ***(Jobe, 2003).***

Postnatally, both inflammatory cytokines and lipid mediator levels are increased in plasma and tracheal aspirates from preterm infants with RDS, and even more so in infants with BPD ***(Groneck et al., 1995).***

These mediators induce a massive pulmonary influx of neutrophils. Neutrophils cause local pulmonary injury and increase alveolocapillary permeability, leading to the clinical picture of respiratory distress ***(Ferreira et al., 2000).***

Inflammation also plays a major role in the pathogenesis of acute respiratory distress syndrome (ARDS) and chronic obstructive pulmonary diseases (COPD) in adults. The anti-inflammatory protein, Clara cell protein, has been extensively investigated in these lung diseases ***(Broeckaert et al., 2000).***

The Clara cell protein has been studied in a wide variety of species, including rats, mice, rabbits, dogs and humans. Depending on the species and the source of isolation, it has

been referred to in the literature by various names, including uteroglobin (UG), human protein 1, urine protein 1, polychlorinated biphenyl (PCB)-binding protein, Clara cell secretory protein (CCSP), Clara cell 10 kD protein (CC10) and Clara cell 16 protein (CC16). The exact molecular mass of this protein, as determined by electrospray ionization/ mass spectrometry, is 15,480, which justifies the abbreviation of CC16 used hereafter to designate the protein ***(Hermans et al., 1999).***

CC16, one of the major secretory proteins of the lungs, is produced by Clara cells along the tracheo-bronchial tree ***(Broeckaert et al., 2000).*** CC16 has many anti-inflammatory properties, which include inhibition of the production of pro-inflammatory cytokines and neutrophil infiltration ***(Harrod et al., 1998).***

Furthermore, CC16 is an important natural inhibitor of the phospholipase A_2 (PLA_2) enzyme ***(Jorens et al., 1995).*** PLA_2 catalyses the rate-limiting step in the formation of inflammatory lipid mediators, collectively called eicosanoids, by hydrolyzing phospholipids and releasing their precursor arachidonic acid ***(Yedgar et al., 2006).*** The subtype secretory phospholipase A_2 ($sPLA_2$), an acute phase protein, is secreted by many cells, e.g. neutrophils, during inflammatory diseases. Besides its role in eicosanoid metabolism, $sPLA_2$ destroys bacteria by hydrolysing their membrane lipids and disturbs surfactant function through the hydrolysis of surfactant lipids ***(Yedgar et al., 2006).***

In ARDS, the concentration and activity of $sPLA_2$ are increased in the blood and lungs, leading to inflammation and surfactant dysfunction ***(Touqui et al., 1999).***

The concentration of CC16 in bronchoalveolar lavage fluid (BALF) depends on the number and integrity of the Clara cells. CC16 concentrations are increased in ARDS and decreased in the BALF from smokers, patients with COPD and idiopathic pulmonary fibrosis ***(Broeckaert et al., 2000).***

In blood, the concentrations of CC16 reflect alveolocapillary membrane permeability ***(Hermans et al., 1999).*** In adults, serum CC16 is considered to be a sensitive and specific marker of lung injury ***(Broeckaert et al., 2000).***

However, the relationship between serum CC16 concentrations at an early stage of life and the development of neonatal lung disease has not been fully investigated. Furthermore, in the preterm infant, CC16 concentrations have not been related to $sPLA_2$ activity.

AIM OF THE WORK

The aim of this work was to determine the role of cord blood clara cell protein CC16 concentration as a predictor for the development of respiratory distress (RDs) and bronchopulmonary dysplasia (BPD) in preterm infant, and to investigate the relation between CC16, $SPLA_2$ and IL-6.

RESPIRATORY DISTRESS SYNDROME (RDS)

Definition:

Respiratory distress syndrome (RDS), previously known as Hyaline Membrane Disease, occurs predominantly in premature infants. The condition is characterized by signs of respiratory distress and increasing oxygen requirements shortly after birth as a consequence of surfactant deficiency. The criteria for RDS include: oxygen-dependence increasing during the first 24 hours, absence of any sign of infection, a typical radiological appearance with reduced air content, reticulogranular pattern of the lung, and air bronchograms (Figure1). The incidence of RDS is inversely related to gestational age and weight. RDS occurs in 60-80% of babies less than 28 weeks and 15-30% for babies born between 32 and 36 weeks. RDS may be present beyond 36 weeks or at term but is uncommon and other diagnoses should be considered. The rates of RDS have fallen significantly since the introduction of prenatal corticosteroids and prophylactic surfactant however it remains a major cause of morbidity and mortality amongst neonates. Currently approximately 80% of women at high risk of a preterm delivery receive corticosteroids ***(Kramer et al., 2009).***

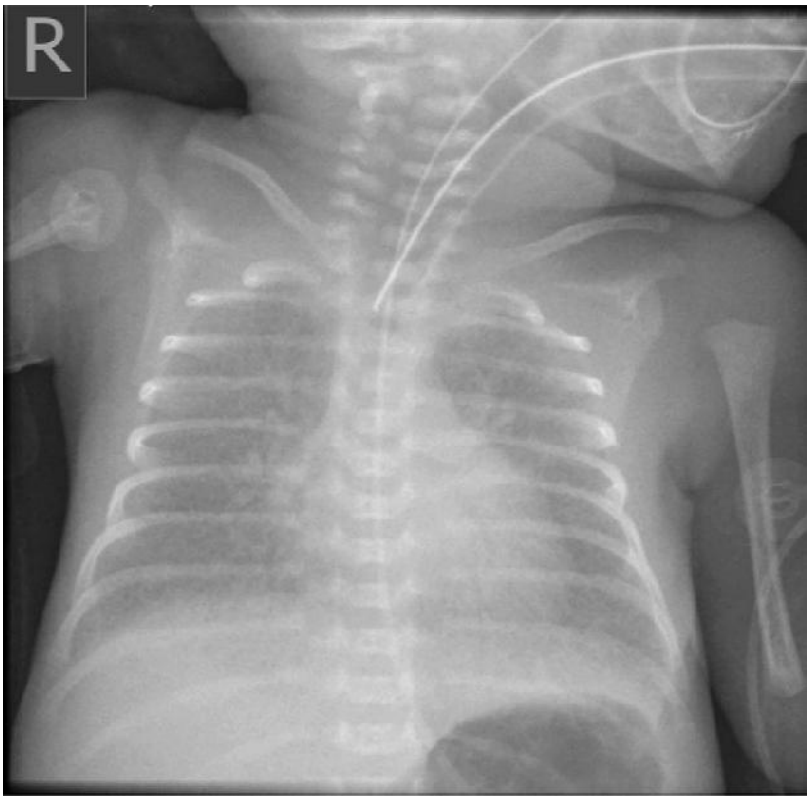

Figure 1: Appearance of RDS on CXR before surfactant administration – the ground glass appearance of the lungs ***(Kramer et al., 2009).***

Risk factors:

Although prematurity is the predominant risk factor for RDS there are several other factors to consider. They are listed in (Table 1). Some of the risk factors are discussed in greater details below.

Table 1: Risk factors of respiratory distress ***(Dani et al., 1999).***

Increased risk	Reduced risk
Advanced maternal age	African origin
Caesarean section delivery	Alcohol intake
Caucasian ethnicity	Antenatal corticosteroid prophylaxis
Congenital diaphragmatic hernia	Chronic/pregnancy associated hypertension
Gestational diabetes	Maternal narcotic addiction
Hypothermia	Prolonged rupture of membranes
Intrapartum asphyxia	Smoking
Male gender	
Meconium aspiration	
Multiple births	
Prematurity	
Family history of RDS	
Pulmonary hypoplasia, infections or haemorrhage	

Prematurity:

The greatest risk factor for RDS is low gestational age and the development of the disease begins with the impaired synthesis of surfactant associated with prematurity ***(Rubaltelli et al., 1998).***

Advanced maternal age:

Dani et al. (1999) were the first to identify an association between RDS and maternal age. Their explanation was based on increased levels of maternal diseases (hypertension, diabetes, placenta praevia and placental abruption) in older women.

Caesarean section:

Caesarean sections increase respiratory morbidity in neonates. Hansen et al. demonstrated a twofold to fourfold increase in respiratory morbidity for babies born by elective caesarean section at 37-39 weeks' gestation. The reason for this is likely to be due to a delayed removal of lung fluid and the lack of a stress response which occurs during spontaneous labour.

Ethnicity:

Infants of African origin have a reduced susceptibility to developing RDS, compared to Caucasian infants. However, there is no significant difference between Caucasian and Caribbean infants. The explanation for this finding is advanced lung maturation amongst African babies. Higher lecithin: sphingomyelin (L:S) ratios related to gestational age have also been reported in certain black infants ***(Kavvadi et al., 1998).***

Diabetes and RDS:

Robert et al. (1976) published their findings of an association between maternal diabetes and RDS in 1976. The explanation for this is that insulin has been shown to inhibit the accumulation of surfactant protein A (SP-A) and surfactant protein B (SP-B) messenger RNA. However, recent evidence has suggested that with modern antenatal care and good follow-up diabetes mellitus, whether

pre-gestational or gestational does not seem to pose any additional risk of RDS to infants born at very low birth weight. However, after 34 weeks the evidence is of the contrary and suggests gestational diabetes to be an independent risk factor for severe respiratory failure ***(Vignoles et al., 2011).***

Narcotic usage:

It has been established since the 1970's that maternal narcotic use reduces the incidence of RDS amongst exposed babies. It is thought that the exposure to narcotics induces lung maturation.

Chorioamnionitis:

Early evidence suggests that chorioamnionitis protects against RDS as inflammation enhances lung maturation improving surfactant production and gas exchange. However, recent evidence has shown that RDS occurring in combination with chorioamnionitis leads to the production of overwhelming inflammatory mediators resulting in surfactant deficiency or inactivation ***(Lee et al., 2011).***

Gender:

Boys are more likely than girls to develop RDS (male-to-female ratio~1.3:1) ***(Dani et al., 1999).*** These differences are thought to be partly due to androgenic

actions on type II pneumocytes delaying the production of mature surfactant ***(Rodriguez et al., 2001).***

Multiple pregnancy:

In twin pregnancies, the second twin is usually at greater risk of developing RDS. This risk of developing RDS in the second twin increases with gestation and is most significant after 29 weeks. It is not clear whether this increased risk is due to delayed maturation of the lungs or an increased risk of hypoxia/acidosis in the second twin ***(Hacking et al., 2001).***

Genetic disposition:

Cases of familial RDS in term babies have been reported and it is now clear that some of these are due to genetic reasons, such as partial or complete deficiency of SP-B ***(Nogee et al., 2004)***. In cases where SP-B is completely absent, death is inevitable despite intensive care and surfactant treatment. Partial deficiency of SP-B has also been reported and this may be compatible with survival. Similar genetic defects of other components of surfactant are increasingly being described ***(Hamvas et al., 2007).***

Intrahepatic cholestasis of pregnancy:

It has recently been shown that maternal intrahepatic cholestasis of pregnancy is significantly associated with the

occurrence of RDS in the newborn. It has been hypothesized that bile acids can cause surfactant depletion in the alveoli ***(Zecca et al., 2006).***

Other risk factors:

Secondary surfactant deficiency may occur in infants with intrapartum asphyxia, pulmonary infections (e.g. Group B β-haemolytic streptococcal pneumonia), pulmonary haemorrhage, meconium aspiration syndrome, congenital diaphragmatic hernia or pulmonary hypoplasia. RDS is further exacerbated by treatable and preventable factors, including hypothermia, hypoxia and acidosis, which impair surfactant production and secretion.

Fetal stages of lung development:

By understanding the embryology of lung growth and development one can appreciate why and how RDS develops. The four main stages of lung development are embryonic, pseudoglandular, canalicular and alveolar. (Figure 2) summarize these stages.

Embryonic stage (0-7 weeks):

The lung begins as a ventral diverticulum from the endodermal foregut in the 4th week after ovulation. Divisions occur and the main hilar connections of the airways and the pulmonary circulation are made. The left and right bronchi are developed by 26-28 days and

segmental airways develop by 6 weeks. Further airway divisions occur leading to the pseudoglandular phase.

Pseudoglandular stage (7-17 weeks):

During the pseudoglandular phase further divisions of the airways occurs. By the end of this stage all pre-acinar airways to the level of the terminal bronchioles are formed.

Canalicular phase (17-27 weeks):

During the canalicular phase the blood-gas barrier thins and the surfactant producing system develops. The peripheral airways continue to divide to form respiratory bronchioli and beyond these the alveolar ducts. By 20-22 weeks of gestation type I and II alveolar epithelial cells are present lining all saccular air spaces. This is 4-5 weeks before surfactant can be detected in the amniotic fluid. By the end of this stage the air to blood barrier is thin enough to enable gas exchange within the saccules. This stage is key to enabling an extremely premature to survive with the likelihood of moderate to severe RDS.

Saccular stage (28-36 weeks):

During the last trimester whole clusters of sacs form on the terminal bronchioli. At this stage the gas-exchanging surface area increases as the airways wall thins out. Lamellar bodies, containing surfactant and phospholipid, in

type II pneumocytes increase and further maturation of type II into type I cells occurs. Capillaries are closely associated with the type 1 pneumocytes enabling a reduction in the distance between the future air blood interface. By 34 weeks gestation mature cup-shaped alveoli line the elongated saccules which are now termed alveolar ducts.

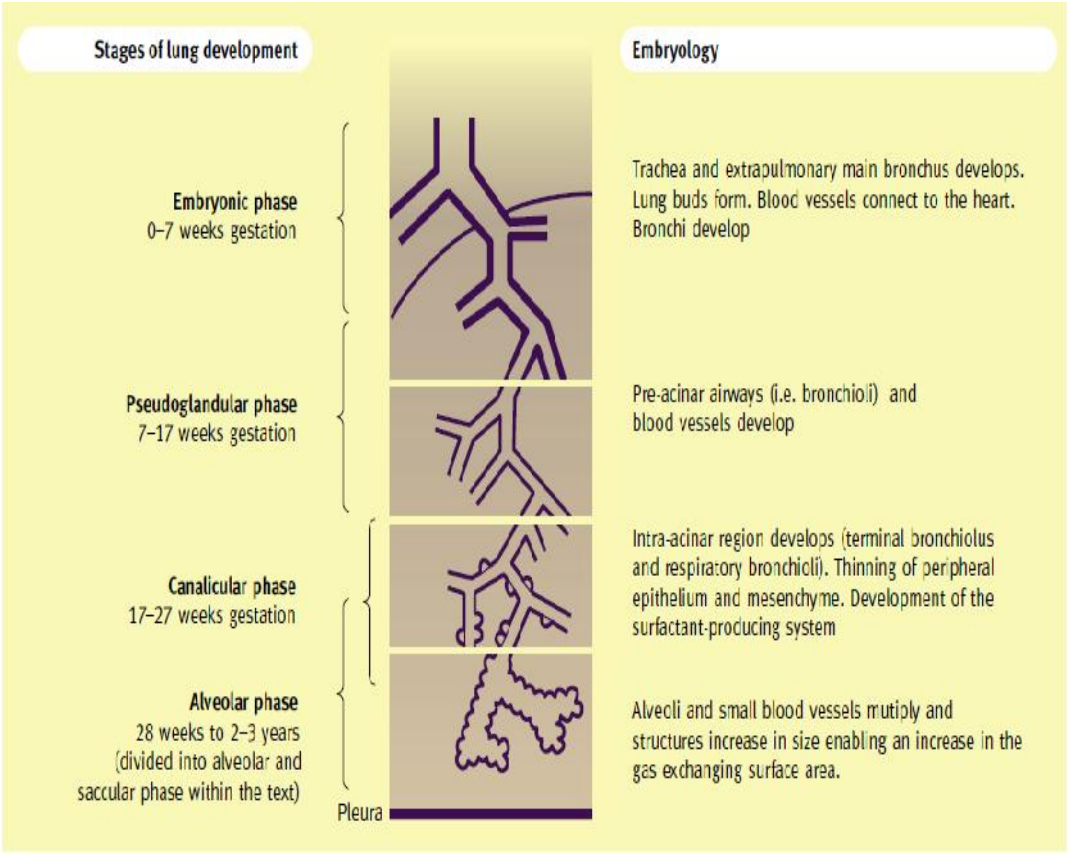

Figure 2: Stages of lung development ***(Kramer et al., 2009).***

Alveolar stage (36 weeks-2 years):

The alveolar stage occurs from 36 weeks gestation until at least 24 months. Alveolar formation and maturation occurs. At the beginning of the alveolar stage the walls of these alveoli are still thicker than in the adult with a double capillary supply and mesenchymal tissue between the epithelial layers. During this phase apoptosis occurs to enable a single capillary loop to develop. The type I and II pneumocytes increase in numbers dramatically to line the alveolar walls. The overall achievement of this stage is an increase in gas-exchanging surface area. It is difficult to estimate the number of alveoli at the different stages but ***Kotecha*** quotes 20-50 million alveoli at birth and approximately 300 million by adulthood ***(Edward et al., 2012).***

Development of type II pneumocytes:

Type II pneumocytes are at the centre of surfactant production and function. Flattening of the acinar epithelium at 22–24 weeks marks the initial differentiation of type II pneumocytes, from which type I pneumocytes will be derived later ***(Kotecha, 2000)***. Type II pneumocytes have a cuboidal shape and comprise 10–15% of the cells of the mature distal lung. They produce surfactant and their characteristic feature is the lamellar bodies which store

surfactant. Lamellar bodies are first observed between 22 and 24 weeks' gestation. Surfactant is secreted from these cells by exocytosis into the lining of the alveoli and appears in the future air spaces at 23–24 weeks' gestation. Type II pneumocytes mature more rapidly between 32 and 36 weeks' gestation, thereby promoting functional maturity of the lung. Type II cells are also important in maintaining structural integrity of the pulmonary alveolus as they proliferate after lung injury and serve as precursors for gas-exchanging type I cells. Type I pneumocytes are flat and elongated and cover the majority of the alveolar surface. Their shape is designed to aid effective gas exchange. These cells are however vulnerable to oxidant damage, that is due to hyperoxia because of their large surface area and reduced anti-oxidant capacity compared to type II alveolar cells which are more resistant to such injury ***(Kotecha, 2000).***

Surfactant:

RDS is the direct consequence of surfactant deficiency. Surfactant has three predominant roles; to increase pulmonary compliance, to prevent atelectasis at the end of expiration and to facilitate recruitment of collapsed airways. In addition surfactant has a role in protecting the lungs from injury and infection caused by foreign bodies and pathogens. Surfactant is synthesized and