Medical Studies Department for Children

Surfactant Protein B Allelic Polymorphism and RDS in Preterm Neonates

Thesis

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By

Manal Shaaban Kishk
M.B., B. Ch; M.Sc. in Childhood Studies

Supervised by

Prof. Khaled Hussein Taman
Professor of Pediatrics
Institute of Postgraduate Childhood Studies - Ain Shams University

Prof. Howida Hosny EL- Gebaly
Professor of Pediatrics-Vice D. Research
Institute of Postgraduate Childhood Studies - Ain Shams University

Dr. Nihal Saad EL- Kinawy
Consultant of Clinical and Chemical Pathology
Ain Shams University

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List of Abbreviations

ABCA3..... adenosine triphosphate (ATP)-binding cassette member A3

ANP ..... Atrial naturetic peptide

ATP ..... adenosine triphosphate

BPD ..... bronchopulmonary dysplasia

C..... cysteine

CPAP ..... Continuous positive airway pressure

CS..... cesarean section

DPPC ..... dipalmitoyl-phosphatidyl-choline

ELBW..... Extremely low birth weight.

ER..... endoplasmic reticulum

ETT ..... endotracheal tube

FRC ..... functional residual volume capacity

GM-CSF..... Granulocyte macrophage colony stimulating factor

HFOV ..... high-frequency oscillatory ventilation

IM ..... Intramuscular

IPPV..... intermittent positive pressure ventilation

IUGR..... Intra uterine growth retardation.

IVH..... intraventricular hemorrhage

L/S..... Lecithin/ Sphingomyelin

LBW ..... Low birth weight.

MAS ..... meconium aspiration syndrome

mRNA..... messenger RNA

MV..... mechanical ventilation

NBW..... Normal birth weight.

List of Abbreviations

NEC ..... necrotizing enterocolitis
NICU ..... Neonatal Infant Care Unit
NIPPV ..... Nasal Intermittent Positive Pressure Ventilation
PC ..... phosphatidyl choline
PCR ..... Polymerase Chain Reaction
PDA ..... patent ductus arteriosus
PEEP ..... Peak end expiratory pressure
PG ..... Phosphatidylglycerol
PHM ..... Pulmonary Hyaline Membrane.
PIE ..... pulmonary interstitial emphysema
PPHN ..... persistent pulmonary hypertension
RDS ..... Respiratory Distress Syndrome
ROP ..... retinopathy of prematurity
Sat PC ..... saturated phosphatidylcholine
SNP ..... single nucleotide polymorphism
SO2 ..... oxygen saturation
SP ..... Surfactant protein
SP-A ..... Surfactant protein A
SP-B ..... Surfactant protein -B
SP-C ..... Surfactant protein C
SP-C ..... Surfactant protein C
SP-D ..... Surfactant protein D
SVR ..... Systemic Vascular Resistance
TTN ..... transient tachypnea of the newborn
UTR ..... untranslated region
VLBW ..... Very low birth weight.

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ABSTRACT

Pulmonary Surfactant components play important roles in normal lung function. SP-B gene is known to be polymorphic and the presence of polymorphisms has been implicated in RDS. **The aim of this study** was to elucidate the association between C1580T SP-B gene polymorphism and RDS among preterm neonates with special emphasis on its impact on clinical status of the disease, severity and outcome. The possibility of using it as a marker in population and family base association studies. **Subject & methods:**40 RDS preterm neonates ,40 healthy preterm controls and 40 fullterm neonates controls were genotyped using Allelic discrimination assay of SP-B C1580T by polymerase chain reaction (PCR) and Restriction enzyme fragment length polymorphism (REFLP). **Results:** The frequency of C allele of SP-B was significantly higher in RDS patients than in controls with an OR=4.5, CI=2.65-7.62, p=0.0001.Genotypic analysis revealed that CC genotype frequencies were higher in RDS patients (37.5%) than preterm controls (12.5%) with a high statistical significant association (P=0.0001). CC genotype is more prevalent in males than females and in those with positive family history showing a statistical significant association ($p<0.05$). Moreover, it was significantly associated with disease severity (p=0.04) and with adverse outcome (p=0.02).While TT and CT genotypes were more prevalent among controls with a high statistical significant association (p=0.0001).**Conclusion:** Higher frequency of genotype CC among RDS patients suggesting that genotype CC might be associated with risk for RDS development and might serve as biomarker of susceptibility of RDS.

Key words: Respiratory distress syndrome, SP-B, Polymorphism, REFLP.

INTRODUCTION

Respiratory distress syndrome (RDS) is a common, devastating, clinical syndrome of acute lung injury. It constitutes one of the main causes of respiratory morbidity in children less than1 year of age despite the improvement in neonatal survival rates. Of the newborns affected by RDS, 5%-25% will develop chronic pulmonary diseases **(Reddy and Kleeberger, 2009).**

RDS occurs primarily in premature infants; its incidence is inversely related to gestational age and birth weight**.** It occurs in 60-80% of infants less than 28 wk of gestational age, 15-30% of those between 32 and 36 wk, about 5% beyond 37wk, and rarely at term **(Christian et al., 2007 and Levit et al., 2009).**

Clinical, epidemiological, and biochemical evidences had strongly suggested that RDS is a multifactorial and mutagenic disease **(Haataj and Hallman, 2002 and Sweet and Halliday, 2009)** and that pulmonary surfactant deficiency is a major factor in the pathophysiology of RDS **(American Lung Association, 2009).**

RDS is principally associated with developmental deficiency in synthesis, intracellular processing, and secretion of pulmonary surfactant **(Marttila et al., 2003 and Pfister et al., 2009).** The Pulmonary surfactant is a unique phospholipids and

protein complex that is synthesized, packaged, and secreted by alveolar type II cells **(Hartl and Griese, 2005).** The phospholipids components constitute (~90%) of pulmonary surfactant, of which phosphatidylcholine (pc) is the most important, accounting for 70-80% of the total **(Brasch et al., 2006).**

The surfactant protein (SP) component constitute (~10%) of pulmonary surfactant. SP plays a fundamental role in the structure, function, and metabolism of the surfactant. SP is required for adaptation to air breathing after birth reducing the surface tension at the air liquid air interface in the alveoli to maintain lung volumes during the respiration cycle. Surfactant protein-B (SP-B) lowers surface tension and prevents atelectasis at end-expiration **(Jeffrey et al., 2004 and Soll and Ozek, 2010).**

SP-B is a 79-amino acid, hydrophobic protein encoded by a 9. 7 Kilo base gene. It is necessary for the formation of lungs and tubular myelin. **(Lahti et al., 2004 and Hamvas et al., 2009).**

Deficiency in SP-B is associated with an alteration in the production of normal lamellar bodies and blocking surfactant secretion causing progressive surfactant dysfunction and lethal respiratory failure **(Garmany et al., 2008 and Wert et al., 2009).**

Several studies have evaluated the association of SP-B gene polymorphisms with RDS, but the functional consequences of the allelic variations of the SP genes are not well understood, and clarification of the genetic diversity is a challenge for the future **(Floros et al., 2001, Wang et al., 2003, Gong et al., 2004 and Lyra et al., 2007).**

The study of the genetic variation of surfactant proteins can help to understand individual variability to develop certain pulmonary pathologies. These genetic variants can serve as valuable markers in the mapping of several diseases particularly RDS and bronchopulmonary dysplasia **(Hamvas, 2010).**

AIM OF THE STUDY

The present study is carried out to elucidate the association between SP-B gene polymorphism and RDS among preterm neonates with special emphasis on its impact on clinical status, severity and outcome.

RESPIRATORY DISTRESS SYNDROME

DEFINITION:

Respiratory distress syndrome (RDS) of newborn, previously called hyaline membrane disease, is a syndrome in premature infants caused by developmental insufficiency of surfactant production and structural immaturity in the lungs. It can also result from a genetic problem with the production of surfactant associated proteins **(Rodriguez et al., 2002).** The term RDS has come to represent the clinical expression of surfactant deficiency and its non, specific histologic counterpart, hyaline membrane disease **(Agrons et al., 2005).**

HISTOLOGICAL BACKGROUND:

In 1903, an initial description of Pulmonary Hyaline Membrane (PHM) was proposed. It was considered by Hochheim to represent aspirated amniotic fluid. In 1923, the first English description of PHM in association with neonatal pneumonia was published. In 1950, an interval of air breathing was proposed as prerequisite to development of PHM, which are especially associated with prematurity, fetal anoxia, maternal diabetes, and cesarean section **(Boyd, 2004)**. A description of the clinical presentation of respiratory

abnormalities in association with PHM was made. In 1951, PHM was viewed to result from tissue damage and transudation of plasma protein and are therefore secondary phenomena; atelectasis was proposed as the significant factor in PHM. Between 1953 and 1955, radiographic descriptions of the reticulogranular pattern in generalized neonatal atelectasis was described and distinguished from the radiographic appearance in cases of aspirated amniotic debris. Between 1953 and 1957, PHM was further attributed to transudation after injury and shown to consist principally of fibrin and entrapped cellular debris **(Negi et al., 2012).**

Perinatal adaptations:

The fetus is prepared during the intrauterine period with normal physical development and neuromuscular control**.**However, as in all complex processes; there are many opportunities for deviation to occur. The physical and neurologic development of the infant cannot always be established before delivery, and silent events in the Perinatal and antenatal period can alter responses in this cycle. During this transitional phase, an infant should be closely monitored so that abnormalities in adaptation may be recognized and addressed ***(Rohan and Golombek, 2009).***

Failure of the perinatal adaptations to occur results in respiratory distress and arterial hypoxemia. Three common

disorders that cause respiratory distress after birth may emerge as: inadequate clearance of fetal lung fluid, resulting in transient tachypnea of the newborn (TTN); deficiency of surfactant, resulting in RDS, persistant pulmonry hypertension ***(Welty, 2009).***

INCIDENCE AND PREVALENCE:

RDS has been reported in all races worldwide, occurring most often in premature infants of Caucasian ancestry. A recent epidemiological study in the United States estimated that there are 80, 000 cases of neonatal RDS each year, resulting in 8500 deaths and hospital costs in excess of $4.4 billion. Neonatal RDS affects approximately 1% of all live births; however, not all infants are at equal risk. Infants with RDS are nearly always premature, it affects 10% to 15% of all infants with a birth weight less than 2500 g and it remains a major cause of perinatal morbidity and mortality in extremely premature infants **(*Pramanik, 2006 and Wheeler et al., 2015).***

RDS is encountered less frequently in the developing countries than elsewhere, primarily because most premature infants, who are small for their gestation, are stressed in utero because of malnutrition or pregnancy-induced hypertension. Because most deliveries occur at home, accurate records are