



Medical Studies Department for Children

Surfactant Protein B Allelic Polymorphism and RDS in Preterm Neonates

Thesis

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List of Contents

| <i>Title</i> | <i>Page No.</i> |
|---|-----------------|
| List of Abbreviations..... | ii |
| List of Tables | iv |
| List of Figures | v |
| Abstract | vii |
| Introduction | 1 |
| Aim of the work | 4 |
| <u>Review of Literature</u> | |
| • <i>Chapter (1):</i> Respiratory distress in the newborn | 5 |
| • <i>Chapter (2):</i> Pulmonary surfactant | 48 |
| • <i>Chapter (3):</i> Surfactant Protein B | 63 |
| • <i>Chapter (4):</i> SP-B allelic polymorphism and RDS.... | 69 |
| Subjects and Methods | 73 |
| Results | 83 |
| Discussion | 101 |
| Conclusion..... | 111 |
| Recommendations | 112 |
| Summary | 114 |
| References | 118 |
| Appendix | 1-3 |
| Arabic Summary | -- |

List of Abbreviations

| | |
|---------------------|---|
| ABCA3 | adenosine triphosphate (ATP)-binding cassette member A3 |
| ANP | Atrial natriuretic 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. |

List of Tables

| <i>Table No.</i> | <i>Title</i> | <i>Page No.</i> |
|--------------------|---|-----------------|
| Table (1): | Clinical scoring of RDS by Down`s score | 22 |
| Table (2): | Diagnosis of RDS by Down`s score..... | 22 |
| Table (3): | Differential diagnosis of neonatal respiratory distress. | 24 |
| Table (4): | Surfactant preparation 2010. | 42 |
| Table (5): | Complications of mechanical ventilation..... | 46 |
| Table (6): | Changes in Surfactant with Lung Development recycling the phospholipid components of surfactant..... | 61 |
| Table (7): | The demographic characteristics of the RDS patients compared with the preterm and full term controls. | 83 |
| Table (8) | Comparison between RDS patients and preterm and full term controls as regard clinical data | 84 |
| Table (9): | Comparison between RDS patients, preterm and full term controls as regard laboratory data: | 85 |
| Table (10): | Outcome of the studied patients. | 86 |
| Table (11): | The relation between Sex and Severity, X-Ray grading and fate with RDS patients. | 87 |
| Table (12): | The relation between severity of the disease by Downs` score compared to gestational age, x-ray grading and the outcome of the RDS patients. | 90 |
| Table (13): | Genotypic analysis of SP-B (C1580T) among patients with RDS and preterm controls..... | 93 |
| Table (14): | Genotypic analysis of SP-B (C1580T) between RDS patients and fullterm controls. | 95 |
| Table (15): | The relation between SP-B genotyping and the Sex, Severity, X-Ray grading and the Outcome of RDS patients. | 97 |
| Table (16): | Relation between SP-B genotyping and the family history among patients..... | 100 |

List of Figures

| <i>Fig. No.</i> | <i>Title</i> | <i>Page No.</i> |
|------------------------|---|------------------------|
| Fig. (1): | Histologic appearance of the lungs in an infant with RDS. | 19 |
| Fig. (2): | RDS grade 1: fine reticuloglanular motting. | 30 |
| Fig. (3): | RDS grade 2: mottling with air bronchograms. | 30 |
| Fig. (4): | RDS grade 3: diffuse mottling heart borders are just discriminable with prominent air bronchograms. | 30 |
| Fig. (5): | RDS grade 4: Bilateral confluent opacification of lungs (white lung)..... | 30 |
| Fig. (6): | Composition of surfactant | 49 |
| Fig. (7): | Alveolar life cycle of surfactant..... | 60 |
| Fig. (8): | Magnified View of the Air-Liquid Interface of the Alveolar Space with Formation of Pulmonary surfactant films | 65 |
| Fig. (9): | Typical radiographic appearance of respiratory distress syndrome with reticulogranular infiltrates and air bronchogram..... | 77 |
| Fig. (10): | REFLP for SP-B 1580 (c/t) polymorphism site by Dde I digestion: | 81 |
| Fig. (11): | The outcome of the preterms with RDS. | 86 |
| Fig. (12): | The relation between Sex and severity by Downs' score in RDS patients..... | 88 |
| Fig. (13): | The relation between Sex and X-ray grading among RDS patients..... | 88 |
| Fig. (14): | The relation between Sex and outcome among patients. | 89 |
| Fig. (15): | The relation between severity of RDS and gestational age..... | 91 |
| Fig. (16): | The severity of RDS by Downs' score compared to X-ray grading in RDS patients. | 91 |

List of Figures (Cont...)

| <i>Fig. No.</i> | <i>Title</i> | <i>Page No.</i> |
|-------------------|--|-----------------|
| Fig. (17): | The relation between severity of RDS and the outcome of patients..... | 92 |
| Fig. (18): | Genotypic analysis of SP-B (C1580T) between neonates with RDS and preterm controls. | 94 |
| Fig. (19): | Genotypic analysis of SP-B (C1580T) among RDS patients and fullterm controls..... | 96 |
| Fig. (20): | Relation between SP-B genotyping and the sex of RDS patients..... | 98 |
| Fig. (21): | Relation between SP-B genotyping and severity by the Downs' score among RDS patients. | 98 |
| Fig. (22): | Genotype frequencies of SP-B in RDS patients in relation to X-Ray grading..... | 99 |
| Fig. (23): | Relation between SP-B genotyping and the outcome of patients..... | 99 |
| Fig. (24): | Relation between SP-B genotyping and the family history among patients..... | 100 |

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 than 1 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 have 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 Griesse, 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, persistent pulmonary 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