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PhD THESIS

CLINICAL AND EXPERIMENTAL RESEARCH ON PREVENTION OF NEONATAL RESPIRATORY DISTRESS SYNDROME IN PREMATURE INFANTS

ABSTRACT

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CONTENTS

Key words: prematurity, neonatal respiratory distress syndrome, neonatal respiratory distress syndrome prophylaxis, antenatal corticosteroid therapy, dexamethasone, pathophysiology of newborn respiration, newborn infections, fetal lung maturation, surfactant.

INTRODUCTION

This PhD thesis aims at addressing a current theme, namely Clinical and Experimental Research on the Prevention of Neonatal Breathing Syndrome Syndrome in Premature. This topic is of great interest because neonatal respiratory distress syndrome is one of the most common respiratory diseases in the newborn, especially in premature babies, and is one of the main causes of neonatal morbidity and mortality.

The doctoral thesis consists of 117 pages, according to the PhD thesis elaboration standards at the "Victor Babeş" University of Medicine and Pharmacy in Timişoara. It is structured in two main parts: the general part is on 33 pages and contains theoretical notions regarding the theme approached, and the rest of the pages are represented by personal research.

For a better argumentation and understanding, 39 figures and graphical representations and 33 tables were used. The bibliography contains 143 titles extracted from treatises or specialty journals.

THE GENERAL PART

The general part of the paper is one third of the thesis and is structured in three chapters.

Chapter 1 is divided in two subchapters: Pulmonary Embryology and Respiratory Anatomy.

In the subchapter Pulmonary embryology are presented the stages of lung development, being explained in detail all 5 stages: the embryonic phase, the pseudoglandular phase, the canalicular phase, the sacral phase and the last stage of pulmonary development, respectively the alveolar phase.

This subchapter is important because it is necessary to know the moment in which the prophylaxis of the respiratory distress syndrome and its effects on the immature lung can be performed.

Chapter 2 is divided into three subchapters: Respiratory Physiology, Elements of Newborn Respiratory Physiology and Neonatal Respiratory Distress Syndrome.

The subchapter of Neonatal Respiratory Distress Syndrome presents the etiology of respiratory distress syndrome, pathophysiology, pathological anatomy, clinical signs, paraclinical diagnosis, treatment, complications, progression and prognosis of this disease, which increases neonatal morbidity and mortality, especially in preterm. The more neonatal respiratory distress syndrome is better understood, the sooner the right treatment can be initiated.

Chapter 3 is divided into two sub-chapters: Surfactant and Corticotherapy.

The subchapter Surfactant presents the composition of this lipoprotein, the synthesis and secretion of the surfactant as well as the administration of the exogenous surfactant. Exogenous surfactant administration is very important, especially in preterm less than 32

weeks, immediately after birth, after they have been stabilized, to replace the surfactant that their immature lung is unable to synthesize.

The subchapter Corticotherapy presents the glucocorticoids administered to the antenatal mothers in imminent premature birth. The glucocorticoids used are Betamethasone and Dexamethasone. Administration of antenatal glucocorticoids accelerates fetal lung maturation by increasing the formation and secretion of surfactant and morphological maturation of the lung.

A better understanding of the prophylaxis of neonatal respiratory distress syndrome will help to develop therapeutic strategies and thereby reduce the mortality of this age group.

THE SPECIAL PART

The special part is structured in seven chapters and represents two thirds of the thesis.

Chapter 4 - Premises and purpose of the study

Major advances in today's therapeutic management have made it possible to treat newborns with gestational age up to 24 weeks.

Prenatal administration of steroids to pregnant women at risk of premature birth is the most effective drug intervention. Glucocorticoid therapy helps accelerate fetal lung maturation by increasing production and removal of surfactant. That is why the interest in prophylaxis of premature neonatal respiratory distress syndrome has increased.

The description of standard care in our Third Level Neonatal Intensive Care Unit, as well as the management of premature respiratory distress syndrome prophylaxis, the assessment and treatment of complications, may be useful for future clinical trials to update existing protocols.

Chapter 5 – Objectives

The objectives of the study were to find answers to a number of clinical and epidemiological aspects of prophylaxis of premature respiratory distress syndrome.

By achieving the epidemiological-clinical, experimental and final findings, a contribution was made to clarify some aspects of the factors influencing respiratory distress syndrome in premature babies, the timing of prophylaxis of neonatal respiratory distress syndrome, how it influences the evolution. The pathology acquired in the studied cases, as well as highlighting new perspectives for improvement of prevention in the occurrence of this type of pathology.

Chapter 6 - Material and method of epidemiological and clinical study

The retrospective clinical and statistical study was performed in the County Emergency Clinical Hospital of Timisoara, Neonatology Section, for the period 01.01.2008-31.12.2013, for a period of 6 years. The study included 1045 preterm infants with gestational age under 37 weeks. The inclusion criteria in the study were all premature newborns with gestational age ranging from 24-36 weeks.

The evaluation of premature newborn parameters included:

- demographic variables (sex, background);
- clinical characteristics (gestation age, birth weight, risk factors, Apgar score, infection, treatment at admission, presence of respiratory distress syndrome, complications, ventilator support, surfactant, postnatal development).

The assessment of maternal parameters included:

- mothers' background environment;
- number of pregnancies and births of mothers;
- level of education;
- the occupation of mothers;
- the marital status of mothers;
- performing corticotherapy for the prevention of neonatal respiratory distress syndrome;
- associated maternal pathologies: diabetes mellitus, HTA.

Chapter 7 - Outcomes and discussion of the epidemiological-clinical study

In the thesis I analyzed a total of 1,045 premature who had gestational age below 37 weeks, between 24 -36 weeks and who were hospitalized between 01.01.2008 - 31.12.2013 in Neonatology Department of Clinical Hospital Of Emergency in Timisoara.

The study covers a 6-year interval in which 1045 preterm cases were analyzed to detect the incidence of neonatal respiratory distress syndrome and the benefits of establishing neonatal respiratory distress syndrome prophylaxis in premature infants.

During the 6-year study of the 13976 newborns, 1045 were born prematurely, with gestational age below 37 weeks. The incidence of prematurity in the study period (n = 1045) was 7.5% relative to the total number of births (n = 13.976).

Of the total number of premature neonates in the studied period (n = 1045), 482 showed a RDS, variable forms, which means 46.1%, ie almost half, which is statistically significant because it represents the target group for RDS prophylaxis with prenatal corticosteroids and for surfactant therapy.

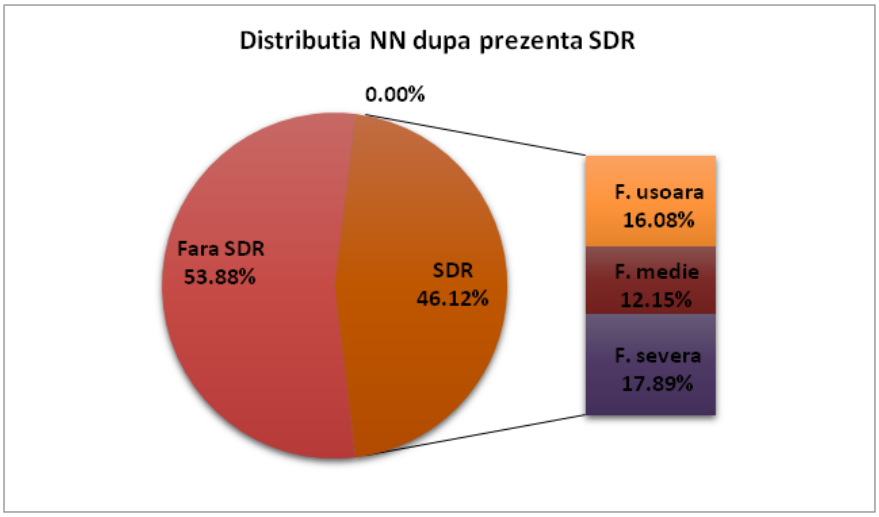

Fig 16. Distribution of newborns according to the presence of RDS

Gestational age (weeks of amenorrhea starting on the first day of the last menstrual period), based on Ballard score, was the basic criterion in assessing fetal maturity / immaturity and delineation of the target group.

As we have mentioned, this group (n = 482) represents the preterm who developed a RDS of varying severity after birth. This category is between the 24-32 week gestational age (n = 407) and 24-33 weeks (n = 553).

As noted, 516 preterm (49.4%) had an IA ≤ 7, indicating moderate to severe adjustment abnormalities. In this logic, 281 preterm (26.9%) had an IA≤6, and 188 preterm (18%) had an IA≤5, which means that half of the preterm had an adjustment disorder from moderate to severe, and this includes the target group, those who developed a SDR.

From the data analyzed, we noticed that premature babies with difficult neonatal adaptation developed very early a severe RDS.

In the Obstetrics-Gynecology and Neonatology Clinic of Timişoara County Emergency Clinical Hospital and in the territory (region), the SDR prophylaxis is according to the guide, with Dexamethasone.

From the data from the study group (n = 1,045 cases), we previously mentioned that 553 preterm (53%) had a gestational age of 24-33 weeks, which is actually the preterm group who could do this prophylaxis.

This was complete in only 130 cases (23.5%) and incomplete in 50 cases (9%). Cumulatively, we can assert that only one-third of premature babies at risk of developing RDS benefit from RDS prophylaxis, which is very little.

Table 21. Incidence of prophylaxis with Dexamethasone

Protocol-DEXAMETAZONA	Number of moms	%
Not done	302	62,65
Complet	130	26,97
Incomplet	50	10,38
Total	482	196,03

This high-efficiency indicator to reduce the incidence of SDR and surfactant should be greatly improved as most studies report an incidence of prophylaxis of over 75-80% or greater.

Of the total number of 13976 newborns in the 2008-2013 period, 1,045 cases were premature newborns, 482 cases had neonatal respiratory distress syndrome and 283 cases had infections.

Table 22. Incidence of RDS and infections in premature neonates

Nr. NN in period 2008-2013 (population)	13976
Preterms in period 2008-2013	1045
Preterms with RDS	482
Preterms with infections	283
Incidence of preterms babies	7.48%
Incidence of preterm newborns with RDS across the population	3.45%
The incidence of infections in preterm infants across the population	2.02%
The incidence of RDS in the premature infant population	46.12%
The incidence of infections in premature infants	27.08%
The incidence of infections in RDS patients in the premature population	58.71%

There is an incidence of premature births in the 6 years of 7.48%. The incidence of premature neonates with respiratory distress syndrome in the whole population was 3.45% and that of newborn infants with 2.02% infection.

We note that the incidence of neonatal respiratory distress syndrome in the premature neonatal population is 46.12%. The incidence of infections in the premature neonate population was 27.08%.

Thus, we can say that prematurity is a risk factor in the occurrence of respiratory distress syndrome and infections.

Infections in premature neonates with neonatal respiratory distress syndrome were 58.71%, a high percentage. Thus, we can conclude that respiratory distress syndrome is a risk factor in the occurrence of infections.

We observe the prevalence of Staphylococcus aureus infections (the most common etiology), followed by coagulase-negative staphylococci, Klebsiella pneumoniae, Serratia and Pseudomonas aeruginosa. Neonatal infections with Candida Albicans are the most common, occupying second position as the incidence of neonatal infections.

A total of 122 preterm newborns needed and benefited from surfactant therapy. If we relate to the total number of cases, we get a small and unimportant percentage. However, in relation to the total number of RDS cases (n = 482), the percentage is 25.3%, which appears to be important. If we refer to the extreme prematurity of 24-32 weeks (n = 407 cases), the percentage is even higher (30%).

We can say that nearly one third of premature babies at risk of developing a RDS have benefited from surfactant therapy. This is related to the national program that only allowed the curative treatment of RDS by the end of the study, not the prophylactic administration of the surfactant immediately after birth.

Chapter 8 - Material and Method in the Experimental Study

Wanting to highlight the effects of corticotherapy on the immature lung by administering a smaller number of doses and at another time before premature delivery, we considered it imperative to carry out an experiment on an animal model (rat), considering changes in the lungs of conception products are studied, which can not be studied on cell cultures.

The experiment was carried out between 01.03.2016 and 01.10.2016, within the Department of Toxicology and Toxicology of the Faculty of Veterinary Medicine of the University of Agricultural Sciences of Banat in Timisoara, with instrumental equipment of micro and macrosurgery, technical and logistic support for handling , anesthesia and contention of experimental animals.

The experiment was performed on white rats. The first stage of the experiment was mating the rats. Stage II of the experiment consisted of male removal and follow-up of gestation (gestation time is approximately 21 ± 2 days). At this stage, Dexamethasone was administered on day 16-18 of gestation.

Chapter 9 - Results and discussion of the experimental study

The protocol of administration of dexamethasone to pregnant women in groups C and B (complete protocol) and lot A (incomplete protocol) was simulated, after which the fetuses were extracted by caesarean and sacrificed at different intervals. After sacrifice, the lung structure was examined and compared to the control structure (fetus born at term and sacrificed).

At the histopathological examination it was noted that the best degree of maturation of lung structures was obtained in rat pups of group C and B (2 doses at 12 hours and 6 hours, slaughter at 12 or 6 hours respectively). This means that in the complete protocol the number of doses of dexamethasone and the interval between administrations before birth is important. In both cases a pulmonary maturation was sensitively approximated to that of mature, term fetus.

From the histoarhitectonic point of view, the fetuses' lungs in group C are at an advanced stage of development, namely the penultimate stage or the sacral stage. The fetal lungs of group B are at an intermediate stage of histoarhitectonic development between the canalicular and the sacral stages.

By comparison, from the histoarhitectric point of view, the fetal lungs of group A fetuses (a dose of dexamethasone administered to the pregnant female 12 hours before birth and sacrifice) are in the penultimate stage of development or sacral stage as in the cases of group C. This demonstrates that in the incomplete protocol a degree of maturation similar to the full one can be achieved provided the prophylactic administration of dexamethasone is done at least 6-12 hours before birth.

Chapter 10 – Conclusions

1. In the 2008-2013 period, 1045 premature newborns were registered at the "Bega" Neonatology Clinic, which represents 7.5% of the total number of births (n = 13976)

2. A total of 482 preterm infants developed a neonatal respiratory distress syndrome (RDS), which represents 46.12% of total preterm infants. This group is in the very low and extremely low premature category (VG <33 weeks) and represents the study group, the rest of the preterm representing the control group (preterm who did not develop a RDS).

3. The study group (n = 482 cases) is also the target group for RDS prophylaxis because this prophylaxis addresses this premature category, and prenatal corticosteroid therapy results in the maturation of the surfactant system.

4. From the recorded data we found that the primary RDS factor at the premature age is the age of gestation and that the degree of immaturity correlates significantly with the risk of premature development of a mean or serious RDS ($p < 0.0001$, CI = 95%) . Along with this determinant we recorded a large number of aggravating factors as follows:

- low and very low birth weight (more precisely in the range 500-1500 g).
- Infectious pathology, very common and almost exclusively interested in the study group with RDS;
- twin pregnancies, very common in the study group, with cumulative risk for each fetus;
- the necessity of reanimation at birth and the introduction of mechanical ventilation in modes and cumulative were recorded almost exclusively in the study group vs. control lot;
- the need for immediate delivery of a surfactant after birth, prophylactically, according to the guidelines, should be the rule for extreme prematurity, very well represented in the study group.

5. Premature babies who developed a SDR at birth (n = 482) are equally the study group and target group that according to obstetrics-gynecology guidelines should benefit from SDR prenatal prophylaxis; Of the recorded data, we found that only 130 mothers of these premature babies (26.97%) benefited from full prenatal corticosteroid protocol.

6. Infectious risk is significantly higher in preterm infants who developed a SDR vs. those without RDS (RP = 1.92, coefficient x 2 = 40.31, p <0.0001, CI = 95%). This risk is significantly increased if the premature baby is mechanically ventilated regardless of ventilation mode, if it has premature rupture of amniotic membranes, if it has received blood and blood derivatives, if it has developed an intracranial haemorrhage and has presented complications of RDS.

7. The recorded data also show that almost ¾ of the cases of the study group did not benefit from prenatal corticotherapy (prenatal prophylaxis RDS) nor prophylaxis due to prophylactic administration of surfactant. This has contributed to the increase in the incidence of RDS, severe forms of illness, the need for mechanical ventilation and the duration of its use, the average length of hospitalization and other parameters that have enormously increased the cost of not applying a cheap and effective prenatal therapy.

8. The experimental researches were carried out with the support of the disciplines of Anatomy, Toxicology and Histopathology of the Faculty of Veterinary Medicine of the Banat University of Agricultural Sciences of Timisoara and aimed at the prophylactic administration of prenatal corticosteroids and its effects on the immature lung; The research was performed on white mouse and rigorously adhered to the standards of experimental research. Its results clearly demonstrate that the prophylactic administration of prenatal dexamethasone, respecting the dosage regimen and the interval between sockets, has therapeutic effect in maturing the lung structures and achieving a histoarchitecture close to that of a normal lung; The structural maturation process occurs even when the administration of corticoids occurs a few hours before birth, so prenatal administration of corticoids is essential in a single dose.

9. Administration of antenatal corticosteroids is quantified by guides and protocols, and its correct application can lead to significant reduction in neonatal morbidity and mortality through prematurity and its respiratory complications. For antenatal corticosteroid administration to have a major impact on neonatal morbidity and mortality, it is necessary to correctly identify and catalog premature birth and pharmacologically useful time (interval).

10. Clinical reality shows that efforts are still needed to implement antenatal measures to prevent SDR in premature babies so as to further reduce the incidence of serious forms of the syndrome, the need for respiratory support, the administration of surfactant and the rate of complications.