

Department of Obstetrics and Gynaecology, Faculty of Medicine

University of Szeged, Hungary

Head: Prof. Attila Pál, M.D., Ph.D.

**SHORT-TERM NEONATAL OUTCOMES FOLLOWING
VARIOUS MODES OF DELIVERY**

Ph.D. Thesis

Zita Gyurkovits, M.D.

Supervisor: Prof. Dr. Hajnalka Orvos, M.D., Ph.D.

Szeged, 2013

INTRODUCTION

Caesarean section (CS) was introduced in clinical practice as a lifesaving procedure both for the mother and for the baby. Surprisingly, the rate of CS increased strikingly and disproportionately in some parts of the world between 1970 and 2009; for instance, the overall rate of CS rose from 6.1% to 31.9% in Hungary, and a similar tendency was seen in the U.S.A., with a rise from 5.5% to 31.8%. The rate of CS is also significantly greater in some high-risk groups, e.g. in preterms, in intrauterine growth retardation (IUGR) or in macrosomia. In the first part of our study, we focused on macrosomic neonates. Secondly, we investigated the possible modification of epidural analgesia (EA) in the process of oxidative stress in neonates born by spontaneous vaginal delivery (SVD), as in cases of SVD the attenuation of labour pain by EA is one of the well-known procedures used worldwide. Finally, in the third part, we established a neonatal lung function test with which we compared lung mechanical parameters in SVD versus CS cases.

Macrosomia

To perform CS in the case of a macrosomic fetus is a dilemma. There is no general consensus as concerns the definition of the term fetal macrosomia. The classical definition for a large-for-gestational-age neonate is a birth weight (BW) more than or equal to the 90th percentile for a given gestational age (GA). Alternatively, the term macrosomia can be utilized for neonates with a BW greater than 4000, 4500 or 5000 g, irrespective of the GA as an absolute limit. The prevalence of macrosomia has been increasing in recent decades, with an accompanying elevated risk of an adverse outcome for mother, fetus and neonate. Fetal overgrowth has both short- and long-term perspectives for both the mother and the neonate. The short-term maternal risks include prolonged labour, perineal lacerations, uterine atonia, abnormal haemorrhage and CS, while the short-term neonatal risks are shoulder dystocia, hypoxia, plexus injuries, hypoglycaemia, and a need for intensive care. Among the long-term risks for the mother, type 2 diabetes mellitus, cardiovascular disease and obesity, and for the neonate cancer have to be mentioned.

Oxidative stress

EA is nowadays a widely-accepted, safe and reliable method of labour pain relief. It has proved to be beneficial to both mother and child, and improves the levels of placental perfusion and oxygenation of the fetus. However, it can be associated with a longer second stage of labour, more frequent oxytocin augmentation, hypotension and fever, due to changes

in the maternal inflammatory reactions, and this may possibly affect the neonatal outcome as well. The normal neonatal physiological responses to the birth process are complex. In particular, shortly after birth, newborns must adapt to abrupt changes in O₂ concentration and to the increased generation of reactive oxygen species (ROS) after their entry into the normoxic environment. The process of birth involves an enhanced degree of oxidative stress for the infant. It is debated whether this stress is a necessary event in the feto-neonatal transition. In 1988, Saugstad conjectured that there may be a link between extreme oxidative stress and neonatal morbidity. Since then, several studies have suggested a connection between oxidative stress and various neonatal disorders. A recent microarray analysis indicated that healthy term fetuses prepare for their impending transition with highly expressed levels of several antioxidant enzymes and associated pathways.

Lung function techniques

One of the greatest challenges facing a newborn after birth is the task of making a smooth transition to air breathing. For the effective gas exchange to occur, the alveolar spaces must be cleared of excess fluid and the pulmonary blood flow increases to match ventilation with perfusion. Failure of either of these events can harm the neonatal transition and cause the infant to develop respiratory distress. It is clear that besides Starling forces and the vaginal squeeze, the amiloride-sensitive Na⁺ transport by the lung epithelia through epithelial Na⁺ channels (ENaCs) is the key event in the transepithelial movement of alveolar fluid. A low lung function at birth is known to be a significant risk factor for acute and chronic lung disease throughout life. It is increasingly recognized that adult diseases have their origin in childhood and this is true for respiratory diseases too. Several infant lung function techniques have been developed over the last 30 years with considerable methodological progress and refinement, but most require sophisticated equipment and sedation, and the reference values for the variables are scarce. There is increasing awareness that airway resistance and forced expiratory flows are determined not only by the calibre of the airways, but also by the compliance of the airway wall and the recoil of the surrounding parenchyma, leading to a search for suitable parameters that will reflect these characteristics. The forced oscillation technique (FOT) was developed in 1956 by DuBois et al., who described the application of a series of sinusoidal pressure waves of varying frequencies to the airway opening or the body surface. In contrast with spirometry, which requires high level of subject effort, the FOT requires no more than quiet tidal breathing for short periods of time. This requirement makes

it an ideal lung function test for use in young children in whom active cooperation is difficult to achieve. These qualities of the FOT make it an ideal tool with which to study airway patency during the neonatal period, and to detect the possible differences in lung mechanics between neonates born by SVD and those born by CS.

AIMS OF THE STUDY

The specific objectives of the study were:

- to compare the neonatal outcomes and modes of delivery in macrosomic and normal BW groups and to analyse the macrosomic subgroups (4000–4499 g, and ≥ 4500 g) in detail in order to explore a possible correlation between morbidities, BW and the mode of delivery;
- to determine and compare fetal oxidative stress indices, levels and activities of antioxidants in the cord blood of singleton, full-term neonates of mothers who received EA on request versus normal SVDs without administration of pain control, and to assess the neonatal outcomes;
- to establish respiratory system impedance (Zrs) data with the FOT superimposed on spontaneous breathing in full-term non-sedated newborns born by CS versus those born by SVD, and to compare the impedance spectra of newborns born by CS or by SVD with postnatal maturation in the 3 subsequent days of life.

MATERIALS AND METHODS

Studies on macrosomic infants

This was a retrospective study on singleton pregnancies of women who delivered between 01.01.2008 and 31.12.2009 at the Department of Obstetrics and Gynaecology, University of Szeged, Hungary. The inclusion criteria were a GA at delivery of at least 37 completed weeks and a BW of at least 2500 g. There were two main groups: the first group comprised neonates with BW 2500-3999 g; this was the control group. The second group comprised the neonates weighing at least 4000 g. These neonates were further stratified into two subgroups from the aspect of their BW (4000-4499 g, and ≥ 4500 g) and the diabetic history of the mother. The neonatal outcome was investigated with regard to the following features: the umbilical cord blood pH, the 5-min Apgar score, fracture of the clavicle,

cephalhaematoma, adrenal haemorrhage, neurological disorders, congenital anomalies, hypoglycaemia, hyperbilirubinaemia, respiratory disorders, admission to a neonatal intensive care unit (NICU), mechanical ventilation and perinatal mortality. Statistical analysis was performed by using the chi-square test; a level $p < 0.05$ was considered to be statistically significant.

Comparison of oxidative stress markers in vaginal deliveries with or without epidural analgesia

This prospective study, approved by the Ethics Committee at the University of Szeged, involved a total of 86 singleton infants born by SVD and their healthy, non-smoking mothers. The parturients were of matched mixed parity in active, spontaneous term labour after an uncomplicated pregnancy. The mothers received full pregnancy care. The exclusion criteria included the use of any medication, coexisting diseases, instrumental delivery or CS. The cord blood samples were provided by the Department of Obstetrics and Gynaecology, Medical University of Szeged, Hungary. Eighty-six singleton full-term mature neonates of either sex, born at a GA between 37 and 41 weeks, were selected, 36 in the EA group, and 50 in the control group. Glutathione (GSH) level, superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), lipid peroxidation (LP) assays were determined. Statistical analysis of the data was performed with Student's t-test. A level of $p < 0.05$ was accepted as indicating statistical significance. The Shapiro-Wilks test was applied to confirm the normality of the values. The reported values are means \pm SD.

Neonatal lung function tests

In this prospective study, approved by the Ethics Committee of the University of Szeged, 42 full-term, healthy singleton newborns were recruited at the Department of Obstetrics and Gynaecology, University of Szeged, Hungary, after written informed parental consent had been obtained. The exclusion criteria included any congenital abnormalities, coexisting diseases or the use of any medication. The neonates were divided into two groups: 19 were born by SVD (BW: 3.15 ± 0.42 kg), and 23 by CS (BW: 3.28 ± 0.47 kg). They were studied on the 1st, 2nd and 3rd days of life. The measurements were made by the FOT during quiet sleep at least 30 min after a feed. Oxygen saturation and heart rate were monitored continuously throughout the study. For the measurement of Zrs, low-amplitude (< 2 hPa) pseudorandom forced oscillations in the 8-to-48-Hz frequency range were superimposed on the spontaneous breathing. The instrument employed for the estimation of Zrs included a

wave tube connecting the source of forced oscillations, i.e. the loudspeaker, and the subject. The spectra with the lowest reproducible Zrs values were selected for ensemble-averaging in each subject for each measurement day. The average Zrs data were fitted by a resistance (Rrs) - inertance (Irs) - elastance (Ers) model in the 12-to-32-Hz range (for average Rrs) and 8-to-32-Hz range (for Ers and Irs). The Zrs parameters obtained on the 3 subsequent days in the SVD and CS groups were analysed with 2-way repeated measures ANOVA.

RESULTS

Studies on macrosomic infants

A total of 5738 singleton births were included in the study from the 2-year period; 410 of the newborns were macrosomic, an incidence of 7.1%. The heaviest baby weighed 5500 g; he was born to a non-diabetic mother. Among the mothers of the 410 macrosomic infants, 43 (10.5%) had diabetes: 9 (2.2%) were pre-gestational and 34 (8.3%) gestational diabetes mellitus (GDM). In the control group, 316 (6.6%) mothers had diabetes: 26 (0.5%) were pre-gestational and 290 (6.1%) GDM. The prevalence of maternal diabetes was significantly higher in the macrosomic group than in the control group (10.5% vs. 6.6%; $p < 0.05$). The number of CS cases in the macrosomic group was 202 (49.3%), which was significantly more frequent ($p < 0.001$) than in the control group: 1898 (39.9%). It was a very interesting finding that the male-female ratio among the macrosomic infants was 2.15 to 1, whereas in the control group it was 0.95 to 1. The difference was significant ($p < 0.001$). As concerns the general condition of the macrosomic infants at birth, in 70 (17.0%) neonates the umbilical cord pH was < 7.2 , but most of them showed a quick recovery. Fortunately, only 4 (0.9%) of the 70 neonates had a 5-min Apgar score < 7 . NICU admission was needed for 21 (5.1%) patients, at either secondary or tertiary care; 21 (5.1%) had a respiratory disorder. Hypoglycaemia was found in 25 (6.1%) cases among the macrosomic infants, and 138 (2.9%) in the control group; the difference was significant ($p < 0.001$). The incidence of polycythaemia did not differ significantly. As concerns the incidence of birth trauma, there was no significant difference in clavicle fracture or cephalhaematoma. There was a highly significant difference in the incidence of adrenal haemorrhage: 4 (0.98%) vs. 7 (0.15%); $p < 0.001$. The only parameter which was significantly higher in the control group was the rate of hyperbilirubinaemia: 1446 (30.4%) vs. 76 (18.5%); ($p < 0.001$). In both study groups, the perinatal mortality was zero. Statistical correlation analysis of the 4000-4499 g and ≥ 4500 g

subgroups revealed significantly more cases in the higher weight group as regards an Apgar score <7 at 5 min, clavicle fracture, and NICU admission. In the case of polycythaemia, the correlation was very close to being significant. The macrosomic neonates of diabetic mothers demonstrated a significantly higher incidence of hypoglycaemia, hyperbilirubinaemia and cardiomyopathy than did those of non-diabetic mothers.

Comparison of oxidative stress markers in vaginal deliveries with or without epidural analgesia

The oxidative stress represented by the level of LP was significantly lower in the EA group than in the control group (4.0 ± 1.5 vs. $6.5 \pm 1.8 \times 10^{-2}$ nmol/mg protein; $p < 0.05$). As regards the antioxidants, the concentration of GSH (5.15 ± 0.48 vs. 7.75 ± 0.63 nmol/mg protein) was also significantly lower in the EA group ($p < 0.05$). Of the antioxidant enzymes, CAT exhibited a significantly lower activity (9.65 ± 0.98 vs. $14.08 \pm 1.2 \times 10^{-4}$ BU/mg protein, $p < 0.01$) in the EA group relative to the control group. The levels of SOD (2.68 ± 0.36 vs. 3.2 ± 0.38 U/mg protein) and GP (3.65 ± 0.43 vs. $4.5 \pm 0.52 \times 10^{-3}$ U/mg protein) were non-significantly lower in the EA group. The babies of mothers who received EA demonstrated a significantly lower arterial umbilical cord blood pH and a significantly lower base excess (BE). No cases with a 5-min Apgar score <7 were observed in either group.

Neonatal lung function tests

Seven of 42 newborns were excluded from the study because of nasal congestion ($n=3$), face mask intolerance ($n=3$) or non-correctable leakage around the mask ($n=1$). No adverse events were noted during or following the measurements. In the remaining 35 newborns, Rrs was characterized by a slight initial decrease with frequency, plateauing above 12-20 Hz, and an occasional rise above 32-36 Hz. The mean value of resonant frequency of the respiratory system (f_{res}) was ~20 Hz, most likely reflecting the predominance of high-inertance nasal pathways. The difference in Rrs between the SVD and CS neonates (mean \pm SD: 47.0 ± 20.1 vs. 47.8 ± 22.6 hPa.s/l) was not statistically significant ($p=0.87$). Likewise, there was no significant difference in Ers between SVD and CS (1617 ± 730 vs. 1606 ± 780 hPa/l; $p=0.95$). Rrs and Ers exhibited large day-to-day fluctuations in ~40% of the newborns. In some of these subjects, very high values of Rrs and Ers were occasionally observed on one of the measurement days, whereas on other days their data were much lower and similar to those of the rest of the population. The 2-way repeated measures ANOVA did not reveal any systematic changes in the Zrs parameters during the 3 days. Interestingly, the

pooled values of Ers and Rrs were highly correlated ($r^2=0.742$); this probably indicates unstable lung volumes, including very low ones where both Rrs and Ers are expected to increase. The pooled Irs and Rrs data also exhibited a close relationship ($r^2=0.574$), which suggests that they are linked via the variable geometry of the large airways, and in particular that of the nasal pathways.

DISCUSSION

Studies on macrosomic infants

Our findings regarding the neonatal outcome of macrosomic newborns, e.g. the higher incidence of CS, the male predominance and hypoglycaemia, were similar to those in previous reports; however, we found also that the prevalence of adrenal haemorrhage was significantly enhanced in the macrosomic group. All neonates with adrenal haemorrhage, even in the control group, were delivered by SVD. At our Department, each infant participates in an abdominal ultrasonographic screening programme, which helps provide reliable data on the occurrence of adrenal haemorrhage, even in those cases without symptoms. Adrenal haemorrhage, and especially the bilateral form, needs a further careful follow-up to prevent the later consequences. This finding highlights the importance of the abdominal ultrasonographic screening programme, especially for the macrosomic neonates born by SVD. The macrosomic infants were born in good general condition, without serious birth trauma or brachial plexus paresis, but it should be borne in mind that the proportion of CS was significantly higher in the macrosomic group. Furthermore, there were significantly more complications in the heavier macrosomic subgroup (≥ 4500 g) as concerns a more frequent low Apgar score at 5 min, clavicle fracture and NICU admission. This observation is consistent with the American experience of a sharp increase in adverse neonatal outcome above 4500 g. Neonatal hypoglycaemia is a common problem in macrosomic infants; it is often associated with maternal diabetes. In our study, the incidence of hypoglycaemia was significantly higher in the macrosomic group and among the neonates of diabetic mothers. In this latter group, not only that of hypoglycaemia, but the incidence of hyperbilirubinaemia and cardiomyopathy were also increased. Glycaemic control of the diabetic pregnant is important, as any abnormal elevation of HbA1C increases the risk of teratogenesis. In our macrosomic group, the incidence of congenital anomalies was 4.4%, in comparison with 3.9% in the control group, without a significant difference, showing that these mothers presumably

had undergone appropriate diabetic control. The comparison of the incidence of congenital anomalies between the macrosomic infants of diabetic (9.3%) and non-diabetic mothers (3.8%) resulted in a very close to significant relation ($p=0.1$). Several previous studies have already revealed the most frequent neonatal complications, such as shoulder dystocia, brachial nerve palsy, clavicle fracture and birth asphyxia, but the increased incidence of adrenal haemorrhage among macrosomic newborns born by SVD has not been investigated earlier, to our knowledge.

Comparison of oxidative stress markers in vaginal deliveries with or without epidural analgesia

Red blood cells from neonates born by SVD with the use of EA were found to display significantly less oxidative stress, but they also had significantly lower levels of antioxidant parameters relative to the neonates who underwent SVD without maternal pain relief. On the other hand, these neonates also exhibited a lower cord blood umbilical pH and a lower BE. EA seemed to reduce the level of oxidative stress; however, these neonates more frequently exhibited acidosis with a quick recovery, without consequences in the later neonatal period. The available data regarding the impact of the delivery mode on the level of oxidative stress in the fetal circulation are inconsistent. We presume that elevations in stress and antioxidant parameters are normal physiological responses to the process of birth. This stress could therefore be necessary in the natural process of uncomplicated pregnancies for both the fetus and the mother. In the following, we briefly summarize the most important aspects of this issue. First of all, it has been concluded in several articles that there is a significant relationship between pain and oxidative stress both in animal models and even in preterm infants. Accordingly, pain enhances the level of oxidative stress during delivery in both mother and fetus. This increased level of oxidative stress during delivery naturally induces the antioxidative defence mechanism. As regards the fetus, there is another interesting maturation phenomenon which must be mentioned here. If labour occurs at term rather than earlier, it triggers a compensatory up-regulation of the non-enzymatic antioxidant reserve. This up-regulation could be a benefit of term labour that protects the newborn from the relative hyperoxia at delivery. Numerous articles have investigated the effects of different forms of anaesthesia on the oxidative balance, and the literature seems to agree on the fact that local anaesthetics have a potential antioxidant effect. Nevertheless, interestingly enough, the moderation of stress-induced damage through the administration of EA could also be

beneficial. Preterm or IUGR infants are especially susceptible to ROS-induced damage, since the state of their antioxidant defence is premature, and their ability to increase the synthesis of antioxidants in response to hyperoxia or other oxidant challenges is inadequate. Our results suggest that EA plays a dual role as concerns oxidative stress, tending to attenuate oxidative stress, but also decreasing the level of antioxidants. However, the neonates born to mothers who had received EA manifested acidosis more frequently in the first few minutes of their extrauterine life. Further investigations are definitely needed to evaluate the possible association between the attenuation of oxidative stress and the acid-base balance and on the impact of the modulation of oxidative stress during birth.

Neonatal lung function tests

We present the first study on airway and tissue mechanics achieved by using the FOT in a group of unsedated newborn term infants during quiet sleep. Neither the difference in Rrs nor in Ers between the SVD and CS neonates were statistically significant, however, we concluded that a high success rate could be achieved in the measurement of Zrs in healthy newborns; the Zrs data obtained with this technique are physiologically meaningful and of potential importance in monitoring the lung function in the first few days of life. Pulmonary function tests play a major role in paediatric clinical practice and are of prime importance for diagnosis, monitoring disease progression, and assessing the effectiveness of therapies. Albeit spirometry remains the principal means by which the lung function is assessed in most clinical settings, interest in the use of other, more specific techniques is also growing rapidly. Associations between various FOT parameters and chronic lung disease have already been published in the literature, but we are unaware of any study on healthy term newborns. Since studies on respiratory mechanics in healthy newborns are very scanty, and those obtained with the FOT are apparently unavailable, the Zrs parameters established in the current investigation are difficult to interpret in the context of paediatric respiratory mechanical data. Recommendations have been developed by the American Thoracic Society/European Respiratory Society Working Party and the specific preparation measures for the FOT and details of the methodology have been described. The FOT is currently gaining acceptance in the assessment of paediatric patients, in part because it requires little to no cooperation, which has led to its extensive use in infants and young children. Zrs in the paediatric population has also been reported to have useful diagnostic possibilities in a wide variety of paediatric diseases, including asthma, cystic fibrosis, bronchopulmonary dysplasia, and neonatal lung

diseases. The FOT is particularly promising for use in infants, for whom few alternatives exist for measuring lung function. One of the great advantages of the FOT for assessing the respiratory mechanical function is that it is completely non-invasive. The primary aim of any lung function test is to provide information allowing the accurate separation of healthy subjects and those individuals with underlying airway pathology. Measurements of lung function are feasible in unsedated newborns, the FOT being used to gain important knowledge about respiratory mechanics in the developing lung.

CONCLUSIONS

CS is an effective intervention to reduce maternal and neonatal mortality relative to SVD, when it is medically justified. Newborn and maternal mortality are closely linked, and therefore the risks can be mitigated with quality care during pregnancy, safe delivery by a skilled attendant, and immediate postnatal care, including resuscitation, extra care of low birth weight babies, attention to baby warmth, treatment of neonatal sepsis and early initiation of breastfeeding.

Our research, firstly focusing on macrosomic neonates revealed an expected higher incidence of CS; these macrosomic infants were born in good general condition without serious adverse outcomes. Moreover, we highlighted the increased incidence of adrenal haemorrhage among the macrosomic neonates born by SVD.

Secondly, we compared the levels of oxidative stress in SVD with and without maternal EA, in spite of the fact that the amount of stress which is useful in the process of birth is still unknown.

The third part of our work was to evaluate the lung function in the first few days of life. We presented the first study in Hungary of successful measurements of airway and tissue mechanics in healthy newborns during quiet sleep, which could be helpful in future in the earlier detection of pulmonary abnormalities, but further studies are definitely needed.

LIST OF PUBLICATIONS RELATED TO THE SUBJECT OF THE THESIS

- I. Gyurkovits Z, Kálló K, Bakki J, Katona M, Bitó T, Pál A, Orvos H. Neonatal outcome of macrosomic infants: an analysis of a two-year period. Eur J Obstet Gynecol Reprod Biol 2011;159:289-292. (IF: 1.974)
- II. Gyurkovits Z, Hracskó Zs, Zimányi M, Varga Sz.I, Németh G, Pál A, Orvos H. Comparison of oxidative stress markers in vaginal deliveries with or without epidural analgesia. Redox Rep 2013;18(1):8-11. (IF: 1.732)
- III. Gyurkovits Z, Kálló K, Bakki J, Katona M, Németh G, Pál A, Orvos H. Négyezer gramm és afeletti születési súllyal világra jött újszülöttek neonatológiai adatainak elemzése. Magy Nőorv Lapja 2013;76(1):4-8. (IF:-)

ACKNOWLEDGEMENTS

First, I wish to express my special thanks to my Ph.D. tutor, Professor Hajnalka Orvos, M.D., Ph.D., who has continuously encouraged and supported me with her professional and whole-hearted guidance during my clinical and research activities.

I am truly grateful to Professor Zoltán Hantos, Ph.D., D.Sc. for his scientific support and constructive advice during the planning and execution of the forced oscillation technique used in the neonatal lung function testing.

I am indebted to Professor Attila Pál, M.D., Ph.D., Director of the Department of Obstetrics and Gynaecology at the University of Szeged, and Professor György Bártfai, M.D., D.Sc., Director of the Reproductive Health Ph.D. Programme, for all their help.

My thanks are due to Ilona Sz. Varga, Ph.D. and Zsuzsanna Hracskó, Ph.D, from the Department of Biochemistry and Molecular Biology at the University of Szeged, for their assistance in the laboratory work.

I would like to thank my colleagues and the staff of the Newborn Unit at the Department of Obstetrics and Gynaecology for their support, which is greatly appreciated.

Last, but not least, I thank my family for all their help and encouragement.