POLISH GYNECOLOGY

GINEKOLOGIA
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THE OFFICIAL JOURNAL OF THE POLISH GYNECOLOGICAL SOCIETY

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ORIGINAL PAPER / OBSTETRICS

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ABSTRACT

Objectives: Fetal growth restriction is associated with chronic fetal hypoxia, poor perinatal outcome and increased perinatal mortality. There are no reliable methods to detect cell damage in the central nervous system (CNS) in these patients. The findings of increased an acidic calcium-binding protein (S100B) concentration in biological fluids of infants after brain injury have supported the use of S100B as a biochemical marker of CNS damage. The purpose of the study was to assess blood S100B concentrations in small for gestational age (SGA) and appropriate for gestational age (AGA) newborns and to evaluate the usefulness of S100B for early detection of hypoxia.

Material and methods: The investigation was carried out between November 2011 and April 2014. Serum S100B protein level was assessed in cord blood collected from newborns after birth. Medical records of mothers of neonates studied were reviewed for pregnancy induced hypertension (PIH), preeclampsia, maternal smoking during pregnancy and abnormalities in umbilical artery (UA) Doppler ultrasound examination.

Results: The study was carried out in 88 SGA neonates and 80 AGA neonates. The median value of S100B protein concentration in the SGA study group was significantly higher than in AGA controls ($p < 0.001$). Cord blood serum S100B concentration in SGA neonates with prenatal normal UA Doppler ultrasound findings ($n = 32$) did not differ from that SGA neonates with abnormal prenatal UA Doppler findings ($n = 25$) ($p = 0.74$), but was significantly higher than in AGA newborns ($p < 0.001$).

Conclusions: Elevated S100B protein levels in cord blood collected from SGA newborns may be helpful in detecting infants at higher risk of postnatal neurologic disturbances at an early stage.

Key words: S100B protein; biomarker; SGA; FGR, newborn; fetal hypoxia; CNS; neurological damage

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INTRODUCTION

Small for gestational age (SGA) neonates have been defined as those with birthweight below a threshold, the 10th centile. Infants who are SGA may suffer intrauterine fetal growth restriction (FGR). FGR is defined as a persistent suppression of fetal growth potential that occurs in response to a decrease in oxygen and nutrients supply from the mother to the fetus. Clinical evidence suggests that feto-placental insufficiency and fetal pre-exposure to decreased oxygen is associated with chronic fetal hypoxia and increased perinatal mortality [1]. FGR is associated with poor perinatal outcome, perinatal brain injury and is the strongest risk factor for an unexplained intrauterine death [2, 3]. About 15% of infants with FGR develop some degree of neurological damage [4, 5]. Most SGA/FGR fetuses remain unnoticed until birth, even when routine third-trimester ultrasound is performed [6]. However, not all fetuses measuring less than the 10th percentile for their gestational age are at risk for adverse outcomes and might be constitutionally small. Available diagnostic tools and routine procedures used when hypoxia is suspected (blood pH, lactate levels, cerebral ultrasound) may not be able to detect cell damage and subclinical lesions in the nervous system. Recently, novel potential biomarkers of brain injury and hypoxia have been identified [7–9]. One of them, S100B is an acidic calcium-binding protein expressed and released by astrocytes and mainly concentrated in the central nervous system. Secretion of S100B is an early process of the glial response to metabolic injury (oxygen, serum and glucose deprivation) [9–12]. Previous evidence that S100B concentrations are increased in the presence of brain injury suggests that higher S100B levels in pregnancies with FGR reflect fetal chronic hypoxia [7, 13, 14]. Since increased concentration of S100B can easily be found in biological fluids (cerebrospinal fluid, peripheral and cord blood, urine and amniotic fluid), the protein can be used as a biomarker of brain damage in growth restricted newborns [13–18].

The aim of this study was to assess blood S100B concentrations in small for gestational age (SGA) and appropriate for gestational age (AGA) newborns and to evaluate the usefulness of S100B for early detection of hypoxia. We hypothesized that serum levels of the S100B marker might differ in SGA and AGA infants.

MATERIAL AND METHODS

Patients

The investigation was performed in a tertiary referral center for neonatal intensive care. All neonates who were born SGA at the Department of Neonatology, Medical University of Łódź, between November 2011 and April 2014 were enrolled in the study.

Birth weight and head circumference in newborns were measured and percentile were assessed. SGA was defined as birth weight $\leq 10^{th}$ centile. The control group included AGA infants (defined as having birth weight between 10th and 90th centile) delivered consecutively at term. Infants who died at birth, those who had congenital malformations, and children of mothers who refused to participate were excluded. The gestational age of newborns was determined according to the date of the last menstrual period and the first trimester ultrasound exam. Apgar score was determined at five minutes after birth.

SGA newborns were divided according to anthropometric indices into those with symmetrical and those with asymmetrical fetal growth restriction. SGA newborns have been categorized as "asymmetrical" when the birth weight was disproportionally restricted as compared to head circumference and "symmetrical" when both: weight and head circumference were proportionately small. Asymmetrical growth restriction was defined as any birth weight > 1 SD less than the corresponding head circumference.

Medical records of mothers of neonates studied were reviewed for gestational hypertension (GH), preeclampsia, maternal smoking during pregnancy and abnormalities in

umbilical artery (UA) Doppler ultrasound examination. An abnormal pulsatility index PI for UA was defined as above the 95th centile for gestational age for uncomplicated pregnancies. The study was approved by the local ethics committee (approval No. RNN/175/05/KE). Written informed consent was obtained from all mothers of enrolled neonates.

Blood samples

Cord blood was obtained from newborns directly after birth. Serum was obtained by centrifugation at $3000 \times g$ for 5 min and stored at $-70°$ C (for up to 6 months) until the biochemical assay.

S100B Assay

S100B concentrations were quantified by an enzyme-linked immunosorbent assay (ELISA; EIAab Science Co., Wuhan, China), according to the manufacturer's instructions. Samples were analyzed in duplicate and compared with S100B standard. The lower limit of detection of the ELISA for S100B is 1 pg/mL.

Statistical analysis

Descriptive statistics were used to describe characteristics of the study group. Continuous data were presented as means with standard deviations (SD) or median with minimum and maximum values. Categorical variables were described as frequencies and percentages. The Shapiro-Wilk test was evaluated to determine if the data were normally distributed. Group comparisons for normally distributed data were performed using the *t* test. When the normality assumptions were not satisfied, Mann-Whitney U test for continuous variables and the Fisher's exact test for binary variables were used. All analyses were performed with the use of IBM SPSS Statistics for Windows V. 25.0.0 (IBM Corp. Armonk, New York, USA). We considered a two-tailed p value less than 0.05 to be significant.

RESULTS

The study was carried out in 88 SGA neonates and 80 AGA neonates. Birth weight was lower in the SGA group than in the AGA group (p<0.001). There were no significant differences in maternal age, gender characteristics, mode of delivery and cord blood lactate concentration between the SGA and AGA groups. There was statistically significant difference in the Apgar score and gestational age (lower in SGA group) (Tab.1). All SGA infants were above or equal 32 weeks of gestation.

None of the newborns had chromosomal abnormalities or TORCH infection. In the SGA group, maternal smoking during pregnancy, gestational hypertension (GH) and preeclampsia were observed significantly more often than in the AGA group (Tab. 1). Absent end-diastolic flow in UA was found in 13 cases of SGA group with abnormal prenatal Doppler.

Table 1. Neonate and maternal demographic and obstetric characteristics of SGA and AGA group

Characteristic	SGA group	AGA group	P value
Male neonates, **n (%)**	88 (52)	80 (56)	0.64
Mean birth weight, g (SD)	2132 (543)	3491 (366)	**0.001**
Mean gestation, weeks (SD)	37.1 (3.0)	39.1 (1.0)	**< 0.001**
Median Apgar score at 5 min (min–max)	10 (5–10)	10 (8–10)	**< 0.001**
Cord blood lactate, mg/dL (SD)	45.61 (16.1)	45.6 (14.7)	0.99
Mean maternal age, years (SD)	31.1 (5.0)	30.0 (4.6)	0.15
Mode of delivery, **n (%)**			0.13
Spontaneous VD	20 **(23)**	33 **(41)**	
Operative VD	1 **(1)**	0	

Cesarean section	67 (**76**)	47 (**59**)	
Smoking during pregnancy, n (%)	20 (**23**)	4 (**5**)	**0.002**
GH, **n (%)**	22 (**25**)	3 (**4**)	**< 0.001**
Preeclampsia, **n (%)**	5 (**6**)	0	0.06
Antenatal Doppler changes:			
PI > 95th centile in UA, **n (%)**	25 (**28**)	2 (**3**)	**< 0.001**
Absent end-diastolic flow in UA, n (%)	**13 (15)**		

The bold p-values highlight whether the differences observed were statistically significant.

SD — standard deviation; SGA — small for gestational age; AGA — appropriate for gestational age; GH — gestational hypertension; UA — umbilical artery; VD — vaginal delivery

Cord blood serum concentration of S100B protein at birth was significantly higher in the SGA infants than in the AGA group (p < 0.001) (Tab. 2).

UA Doppler examination was performed in 57 out of 88 (65%) mothers of newborns from the study group. Cord blood serum S100B concentration in SGA neonates with prenatal normal UA Doppler ultrasound findings (n = 32, median 69.3 [0.3–463.5]) did not differ from that SGA neonates with abnormal prenatal UA Doppler findings (n = 25, median 44.4 [6.8–1110.2]) (p = 0.74) but was significantly higher than in AGA newborns (n = 80) (p < 0.001) (Tab. 2).

S100B concentration was significantly higher in both SGA with symmetrical (n = 29) and asymmetrical (n = 59) fetal growth restriction than in non-growth restricted AGA neonates (p < 0.001) (Tab. 2).

Table 2. Cord blood S100B concentrations in AGA, all SGA, those SGA that had normal UA PI and SGA newborns with symmetrical growth

	Group	N	Median	Min–max	P value
	AGA †	80	25.7	**0.1–395.8**	
	SGA	88	64.3	**0.1–2025.0**	**< 0.001**
S100B (pg/mL)	SGA with normal UA PI	32	**69.3**	**0.3–463.5**	**< 0.001**
	"Symmetrical" SGA	**29**	**58.5**	**0.3–95.9**	**< 0.001**

The bold P values highlight whether the differences observed were statistically significant. AGA group serves as a reference (†) when compared separately with the SGA group and the SGA with normal UA PI.

AGA — appropriate for gestational age; SGA — small for gestational age; UA — umbilical artery; PI — pulsatility index

Ultrasound examination of central nervous system was performed in all SGA infants within first 48 hours of life. Four out of 88 SGA infants had abnormal RI in the anterior cerebral artery (< 0.6) in the first head ultrasound examination.

We found no statistically significant differences in S100B concentration in terms of the anterior cerebral artery Doppler scanning between SGA newborns with abnormal RI (< 0.6) and normal RI (> 0.6).

DISCUSSION

The study showed that serum S100B concentrations in the SGA infants were higher than in the AGA group. Our findings confirm the previous results of Gazzolo et al. [13], who

studied S100B levels in cord blood from FGR newborn delivered by elective caesarean section and published the first paper about S100B protein and FGR correlation in 2002. In this study higher S100B levels in umbilical blood of FGR newborn were demonstrated.

Since S100B protein, during an active brain injury, is released from a damaged tissue into circulation, its concentration increases at an early stage of hypoxia in both cerebrospinal fluid and cord blood. The best sources for biomarkers are fluids obtained the least invasively and shortly after birth [8]. As S100B protein is highly concentrated in central nervous system, and has a half-life about one hour, is released by kidney tissue and increases in biological fluids at an early stage. Cord blood and urine seems to be a perfect source for further studies of the potential use of S100B measurements in FGR pregnancies and newborns. Gazzolo et al. [19], reported that higher concentrations of S100B were detected in cord blood of FGR fetuses who developed intraventricular hemorrhage after birth. In addition, the authors observed that the highest maternal S100B concentrations were found in group of fetuses with prenatal brain-sparing effect in prenatal ultrasound examination. In another study, Florio et al., found higher S100B concentrations in urine samples taken shortly after birth in FGR newborns compared to matched AGA controls. They noticed highest S100B concentrations in the neonates with abnormal neurologic follow-up but also significantly high in FGR infants with uneventful neurologic follow-up at one week of age [15]. However, no differences in S100B serum concentration of FGR and AGA infants were found in a small study containing 20 infants in each group [16]. In another study, Gazzolo et al. [13], found a correlation between circulating S100B protein and the fetal middle cerebral artery pulsatility index (MCA PI) that might suggest cerebral cell damage in growth restricted fetuses. This study showed no difference in S100B levels between SGA newborns with no abnormalities in prenatal Doppler examination (normal UA PI) and neonates with normal growth (AGA).

In our study, we attempted to investigate whether S100B concentration depends on prenatal hemodynamic disturbances. When SGA newborns were grouped according to normal and abnormal prenatal UA Doppler examination (UA PI > 95th centile), we did not find any differences in serum S100B concentration between either group. In contrast to Gazzolo et al. [13], we observed significantly higher S100B concentrations in SGA newborns with normal Doppler examination compared to AGA newborns. However, the prenatal ultrasound examinations were not performed in all cases and thus these results should be interpreted with caution.

In this study we also divided SGA group according to the traditional biometric birth measurements to those with symmetrical and asymmetrical growth restriction. Factors that cause symmetric growth restriction tend to develop early during fetal life. Since all SGA newborns studied were older than 32 weeks of gestation, we could speculate that beside growth restricted newborns, "symmetrical SGA" group included constitutionally and physiologically small infants. Both "symmetrical" and "asymmetrical" neonates had higher cord blood S100B concentrations compared to AGA neonates but these non-growth restricted infants could not be recognized on a group level.

The importance of biometric as well as functional parameters for identification of FGR was reported by Baschat [20], then the consensus-based definition of FGR was established in the Delphi criteria [21]. In our study we found that assessment of S100B concentration may be helpful in identifying FGR while Doppler findings are normal. Further studies however are needed to confirm our results.

CONCLUSIONS

Increased S100B protein concentration in blood collected from SGA newborns indicates secretion of this marker in response to hypoxia occurring in intrauterine growth

restriction. Examination of cord blood S100B concentration may be helpful in identifying SGA newborns at a higher risk of postnatal neurological sequelae at an early stage while prenatal Doppler examination is normal, standard clinical and laboratory parameters are silent, and an early-stage neurologic follow-up is uneventful.

Disclosure statement

The authors declare that they have no competing interests.

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