in vivo *23*: 139-142 (2009)

Cord Blood Survivin Concentrations in Human Full-term Normal and Complicated Pregnancies

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Abstract. *Background: Survivin (a member of the inhibitors of apoptosis family) is important for fetal development, placental survival and differentiation. Intrauterine growth restriction (IUGR) and fetal macrosomia, due to maternal diabetes mellitus (DM) are associated with excessive and decreased feto-placental apoptosis, respectively. The aim was to study survivin concentrations in cord blood at term in IUGR, large-for-gestational-age (LGA, due to gestational DM) and appropriate-for-gestational-age (AGA) pregnancies. Patients and Methods: Survivin concentrations were determined in 160 mixed arterio-venous cord blood samples from IUGR (n=48), LGA (n=11) and AGA (n=101) singleton full-term infants. Results: No significant differences in survivin concentrations in cord blood were observed between groups. The effect of birthweight, customized centile, gestational age, gender, delivery mode and parity on survivin concentrations was not significant. Conclusion: Survivin concentrations in cord blood at term are independent of intrauterine growth, gender, parity and delivery mode. Thus, they probably do not reflect the disturbances of feto-placental apoptosis expected in IUGR and fetal macrosomia due to gestational DM.*

Apoptosis (programmed cell death) regulates cell proliferation and contributes to tissue and organ homeostasis during development and differentiation (1). Furthermore, apoptosis is important for the development of the normal human placenta (2). Disturbances in feto-placental apoptosis seem to be associated with abnormal pregnancy outcome, including fetal macrosomia due to maternal diabetes mellitus (DM) (3) and intrauterine growth restriction (IUGR) (4, 5).

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Key Words: Fetal macrosomia, fetus, intrauterine growth restriction, survivin, umbilical cord blood.

In this respect, decreased apoptosis in placental cells has been reported in fetal macrosomia due to DM (3). On the other hand, an increased incidence of apoptosis in IUGR-affected fetal membranes has been documented (4-7). Elucidation of the molecular mechanisms involved in the control of apoptosis in the IUGR-affected fetal membranes may provide further insights into the etiology of IUGR (5). Relatively, apoptosis of IUGR placentas has been correlated with the expression of proteins involved in apoptosis and cell turnover (8, 9). However, cord blood levels of apoptosis markers in fetal macrosomia due to maternal DM have not been reported in the literature, while extremely limited data exist regarding IUGR (10, 11).

Survivin (apoptosis inhibitor 4; API 4), a 16.5 kDa a protein recently described as a member of the inhibitors of apoptosis (IAP) family (12), probably exerts its action by inhibiting an apoptotic pathway involving caspases (13). Survivin, prominently expressed in fetal tissues and overexpressed in cancer cells (14), is important for normal fetal development (15). It is also postulated to play critical roles in placental cell survival and cytotrophoblast cell differentiation (16). Nevertheless, the perinatal cellular or extracellular expression of survivin has not as yet been investigated in large-for-gestational-age (LGA) or IUGR pregnancies.

This study was based on the hypothesis that umbilical cord blood concentrations of survivin in IUGR and LGA (due to gestational DM) cases may differ from respective concentrations in appropriate-for-gestational-age (AGA) controls, since the former are associated with excessive (4-7) and reduced (3) feto-placental apoptosis, respectively. This hypothesis is further supported by a recent study demonstrating that the phenotype of peripheral blood leukocytes in arthritis is affected by extracellular survivin (17). Therefore, this report aims to evaluate and compare cord blood concentrations of survivin in IUGR, LGA and AGA pregnancies at term and correlate them with common demographic parameters of the studied infants.

Table I. *Demographic data of AGA, IUGR and LGA infants at birth*

	AGA Mean±SD (Median)	IUGR Mean±SD (Median)	LGA Mean±SD (Median)
Birthweight (g)	3197±296 (3170)	2566±343 (2605)	3781±173 (3850)
Customized centile	39.3±23.4 (33)	5.33±3.8 (5)	94.1 ±3.1 (94)
Gestational age (weeks)	39.0±1.0 (39.1)	38.4±1.4 (38.3)	38.4±0.9 (38.1)
	N(%)	N(%)	N(%)
Gender			
Male	60 (59.4)	23 (47.9)	9 (81.8)
Female	41 (40.6)	25 (52.1)	2 (18.2)
Mode of delivery			
Vaginal	77 (76.2)	24 (50)	6 (54.5)
Cesarian	24 (23.8)	24 (50)	5 (45.5)
Parity			
First	70 (69.3)	31 (64.6)	7 (63.6)
Other	31(30.7)	17 (35.4)	4 (36.4)

Patients and Methods

The Ethics Committee of our Teaching Hospital approved the study protocol. In the time period from January 2007 to June 2007, one hundred and sixty healthy, singleton full-term (mean gestational age: 38.8±1.1, median 38.4 weeks) infants, whose mothers gave informed consent, were included in the study. From the above, 101 were AGA, 48 were asymmetric IUGR (birth weight ≤10th customized centile), and 11 were LGA (birth weight ≥90th customized centile). The gestation related optimal weight computer-generated program was used to calculate the customized centile for each pregnancy (mean 33±29, median 23), taking into consideration significant determinants of birth weight, such as maternal height and booking weight, ethnic group, parity, gestational age and gender (18). Gestational age was estimated using the date of the last menstrual period and early antenatal ultrasound. Birth weight was measured with an electronic scale and was found to be mean±SD 3,048±462 g (median 3,060 g).

Fourteen of the 48 mothers with IUGR offspring presented with pre-eclampsia (19), 16 presented with pregnancy-induced hypertension, 10 suffered from various diseases, such as iron-deficient anemia (4 cases), severe type I DM (2 cases) and hypothyroidism (4 cases). The remaining 8 women were smoking >10 cigarettes/day during the whole duration of pregnancy.

Amniotic fluid was diminished in all IUGR cases. For the evaluation of the amniotic fluid, the largest fluid column on the vertical plane was assessed and was defined as diminished if <2 cm. Placental weights were reduced (20) ranging from 230 to 420 g.

On the other hand, all mothers with LGA offspring presented with gestational DM, controlled by diet, and placentas were large, weighing from 650 to 810 g (20). In the AGA group, mothers were healthy and were either non-smokers or abstained from smoking during pregnancy. Placentas were normal in appearance and weight, ranging from 480 to 621 g (20).

Tests for congenital infections were negative in all women of the three groups and their offspring had no symptoms of intrauterine infection or signs of genetic syndromes.

One- and five-minute Apgar scores were ≥8 in all infants. Ninety-two infants (57.5%) were boys and 68 (42.5%) were girls. One hundred and seven (66.9%) were born vaginally and 53 (33.1%) were born by cesarean section. Parity was the first in 108 (67.5%) cases and in the remaining 52 (32.5%) cases the second or more (Table I).

Mixed arteriovenous cord blood was collected in pyrogen-free tubes. Plasma was separated by centrifugation and was kept frozen at −80˚C until assay. The determination of plasma survivin concentrations was performed by enzyme immunoassay (Human Total Survivin EIA; Assay Designs, Ann Arbor, MI, USA). The minimum detectable concentration, intra- and interassay coefficients of variation were <31.25 pg/ml, 1.85% and 16.7%, respectively.

Statistical analysis. Data regarding survivin concentrations were not normally distributed (Kolmogorov Smirnov test); thus, a log-transformation of the variable representing survivin was performed. Linear regression analysis was applied to examine the possible effect of different independent variables such as gender, mode of delivery, parity, birthweight and centile, using the log-transformed survivin as dependent variable. Spearman's or Pearson's correlation coefficient was used to detect any positive or negative correlations. A $p<0.05$ was considered statistically significant.

Results

No statistically significant differences in survivin concentrations in cord blood were observed between IUGR, LGA and AGA groups.

The effect of group (IUGR, LGA, AGA) (Figure 1), birthweight, customized centile, gestational age, gender, mode of delivery and parity on circulating survivin concentrations was not found to be significant. Additionally, no significant positive or negative correlations were observed between survivin concentrations and all the aforementioned variables. Reference values (median, range) for survivin were 138.49 pg/ml and 71.54-349.89 pg/ml, respectively.

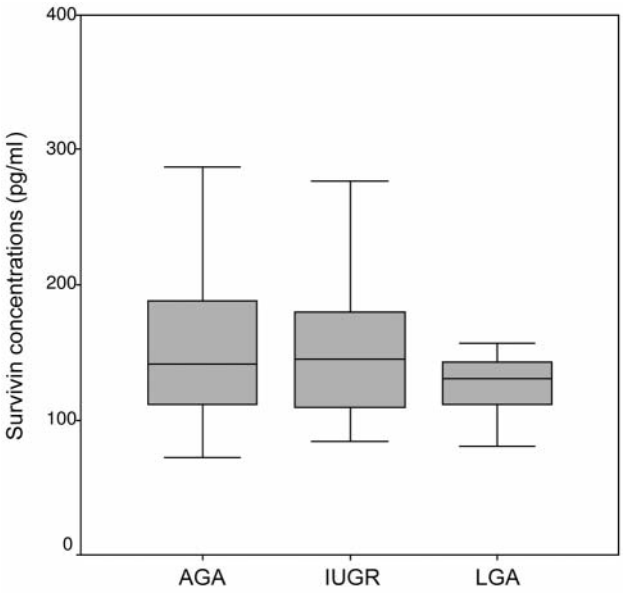

Figure 1. *Cord blood survivin concentrations in AGA, IUGR and LGA infants.*

Discussion

The results of this study indicate a lack of significant differences in survivin concentrations in cord blood at term in IUGR and LGA (due to gestational DM) cases compared to AGA controls.

Birth of a healthy infant at term is dependent upon normal placental function and development (4). Conversely, abnormal placentation is responsible for a wide range of pregnancy complications, including IUGR (21). In asymmetric IUGR, typically caused by uteroplacental vascular insufficiency (22), alteration of the apoptotic process in the uteroplacental unit has been associated with defective placentation (23), which results in increased uteroplacental resistance (4) and reduced placental size at term (5, 24), also recorded in the present study. The consequences of these events in placental development are long term and lead to compromised uteroplacental perfusion and reduced maternofetal transport of oxygen and nutrients (4).

Survivin has a dual role during normal fetal development in apoptosis inhibition and regulation of mitosis (15). It antagonizes apoptosis in cytotrophoblasts and extravillous trophoblasts, acting as a significant regulator of cytotrophoblast cell differentiation (16). Furthermore, it has been previously reported that survivin is a novel growth factor-inducible protective gene expressed by endothelial cells during angiogenesis (25). In this respect, regulation of endothelial cell survival and maintenance of vascular integrity by survivin are crucial for normal embryonic angiogenesis (25, 26). Although data concerning cellular activities may not be directly extrapolated to extracellular ones, in two previous studies no differences in umbilical cord concentrations of angiogenic factors (reflecting angiogenesis) among full-term IUGR and AGA infants were documented (27, 28).

In accordance with the present results, a recent study demonstrated a lack of correlation between apoptosis in the placenta of IUGR pregnancies and birth weight, multiparity, gestational age, gender and mode of delivery (29). On the other hand, exposure to a diabetic milieu decreases apoptosis in placental cells (3). The lack of differences in survivin concentrations in cord blood at term between LGA cases and AGA controls in this study could indicate that survivin concentrations may not correlate with the decreased placental apoptosis expected in fetal macrosomia due to gestational DM.

In conclusion, this study showed a lack of differences in survivin concentrations in umbilical cord blood in IUGR and LGA cases compared to AGA controls. Thus, survivin concentrations in cord blood probably do not reflect the disturbances of feto-placental apoptosis, expected in IUGR and fetal macrosomia, due to gestational DM. Parity, gender and mode of delivery (vaginal or elective cesarean section) do not seem to have any impact on survivin concentrations in umbilical cord blood. Furthermore, the results provide a useful set of survivin reference values in full-term infants at birth. Additional studies, reporting the placental expression of survivin in IUGR and fetal macrosomia, due to gestational DM, are required.

References

1 Vaux DL, Haecker G and Strasser A: An evolutionary perspective on apoptosis. Cell *76*: 777-779, 1994.
2 Ka H and Hunt JS: Temporal and spatial patterns of expression of inhibitors of apoptosis in human placentas. Am J Pathol *163*: 413-422, 2003.
3 Burleigh DW, Stewart K, Grindle KM, Kay HH and Golos TG: Influence of maternal diabetes on placental fibroblast growth factor-2 expression, proliferation, and apoptosis. J Soc Gynecol Investig *11*: 36-41, 2004.
4 Madazli R, Benian A, Ilvan S and Calay Z: Placental apoptosis and adhesion molecules expression in the placenta and the maternal placental bed of pregnancies complicated by fetal growth restriction with and without pre-eclampsia. J Obstet Gynaecol *26*: 5-10, 2006.
5 Murthi P, Kee MW, Gude NM, Brennecke SP and Kalionis B: Fetal growth restriction is associated with increased apoptosis in the chorionic trophoblast cells of human fetal membranes. Placenta *26*: 329-338, 2005.
6 Smith SC, Baker PN and Symonds EM: Increased placental apoptosis in intrauterine growth restriction. Am J Obstet Gynecol *177*: 1395-1401, 1997.
7 Axt R, Kordina AC, Meyber R, Reitnauer K, Mink D and Schmidt W: Immunohistochemical evaluation of apoptosis in placentae from normal and intrauterine growth-restricted pregnancies. Clin Exp Obstet Gynecol *26*: 195-198, 1999.

8 Endo H, Okamoto A, Yamada K, Nikaido T and Tanaka T: Frequent apoptosis in placental villi from pregnancies complicated with intrauterine growth restriction and without maternal symptoms. Int J Mol Med *16*: 79-84, 2005.
9 Levy R, Smith SD, Yusuf K, Huettner PC, Kraus FT, Sadovsky Y and Nelson DM: Trophoblast apoptosis from pregnancies complicated by fetal growth restriction is associated with enhanced p53 expression. Am J Obstet Gynecol *186*: 1056-1061, 2002.
10 Laskowska M, Laskowska K, Leszczynska-Gorzelak B and Oleszczuk J: Evaluation of the maternal and umbilical vein serum sFas/sFasL system in pregnancies complicated by pre-eclampsia with intrauterine growth retardation. Eur J Obstet Gynecol Reprod Biol *126*: 155-159, 2006.
11 Laskowska M, Laskowska K, Leszczynska-Gorzelak B and Oleszczuk J: sCD40 ligand determined in maternal and umbilical cord blood in pregnancies complicated by pre-eclampsia with and without intrauterine growth retardation. Gynecol Obstet Invest *64*: 8-13, 2007.
12 Rothe M, Pan MG, Henzel WJ, Ayres TM and Goeddel DV: The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell *83*: 1243-1252, 1995.
13 Deveraux QL and Reed JC: IAP family proteins – suppressors of apoptosis. Genes Dev *13*: 239-252, 1999.
14 Ambrosini G, Adida C and Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med *3*: 917-921, 1997.
15 Altieri DC: Molecular circuits of apoptosis regulation and cell division control: the survivin paradigm. J Cell Biochem *92*: 656-663, 2004.
16 Shiozaki A, Kataoka K, Fujimura M, Yuki H, Sakai M and Saito S: Survivin inhibits apoptosis in cytotrophoblasts. Placenta *24*: 65-76, 2003.
17 Mera S, Magnusson M, Tarkowski A and Bokarewa M: Extracellular survivin up-regulates adhesion molecules on the surface of leukocytes changing their reactivity pattern. J Leukoc Biol *83*: 149-155, 2008.
18 Gardosi J, Chang A, Kaylan B, Sahota D and Symonds EM: Customized antenatal growth charts. Lancet *339*: 283-287, 1992.
19 CLASP (Collaborative Low-dose Aspirin Study in Pregnancy) Collaborative Group: CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. Lancet *343*: 619-629, 1994.
20 Burkhardt T, Schaffer L, Schneider C, Zimmermann R and Kurmanavicius J: Reference values for the weight of freshly delivered term placentas and for placental weight-birth weight ratios. Eur J Obstet Gynecol Reprod Biol *128*: 248-252, 2006.
21 Kingdom J: Andriana and Luisa Castellucci Award Lecture 1997: Placental pathology in obstetrics: adaptation or failure of the villous tree? Placenta *19*: 347-351, 1998.
22 Laurini R, Laurin J and Marsal K: Placental histology and fetal blood flow in intrauterine growth retardation. Acta Obstet Gynecol Scand *73*: 529-534, 1994.
23 Straszewski-Chavez SL, Abrahams VM and Mor G: The role of apoptosis in the regulation of trophoblast survival and differentiation during pregnancy. Endocr Rev *26*: 877-897, 2005.
24 Kilani RT, Mackova M, Davidge ST and Guilbert LJ: Effect of oxygen levels in villous trophoblast apoptosis. Placenta *24*: 826-834, 2003.
25 O'Connor DS, Schechner JS, Adida C, Mesri M, Rothermel AL, Li F, Nath AK, Pober JS and Altieri DC: Control of apoptosis during angiogenesis by survivin expression in endothelial cells. Am J Pathol *156*: 393-398, 2000.
26 Zwerts F, Lupu F, De Vriese A, Pollefeyt S, Moons L, Altura RA, Jiang Y, Maxwell PH, Hill P, Oh H, Rieker C, Collen D, Conway SJ and Conway EM: Lack of endothelial cell survivin causes embryonic defects in angiogenesis, cardiogenesis, and neural tube closure. Blood *109*: 4742-4752, 2007.
27 Malamitsi-Puchner A, Boutsikou T, Economou E, Sarandakou A, Makrakis E, Hassiakos D and Creatsas G: Vascular endothelial growth factor and placenta growth factor in intrauterine growth-restricted fetuses and neonates. Mediators Inflamm *5*: 293-297, 2005.
28 Malamitsi-Puchner A, Boutsikou T, Economou E, Tzonou A, Makrakis E, Nikolaou KE and Hassiakos D: Angiopoietin-2 in the perinatal period and the role of intrauterine growth restriction. Acta Obstet Gynecol Scand *85*: 45-48, 2006.
29 Erel CT, Dane B, Calay Z, Kaleli S and Aydinli K: Apoptosis in the placenta of pregnancies complicated with IUGR. Int J Gynaecol Obstet *73*: 229-235, 2001.

Received June 2, 2008
Revised November 25, 2008
Accepted December 2, 2008