Elevated Amniotic Fluid Stromal Cell-derived Factor-1α (SDF-1α) Concentration in Mid-gestation as a Predictor of Adverse Birth Outcomes

Jenn-Jhy Tseng[1,2,3*†], Ya-Fang Chen[1†], Yeun-Ting Hsieh[1], Min-Min Chou[1]

[1]Department of Obstetrics and Gynecology, Taichung Veterans General Hospital, [2]Department of Nursing, Hung-Kuang University, Taichung, and [3]Institute of Clinical Medicine, National Yang-Ming University School of Medicine, Taipei, Taiwan, R.O.C.

Background: This study aimed to predict maternal and neonatal outcomes by measuring mid-trimester amniotic fluid stromal cell-derived factor-1α (SDF-1α) concentration in healthy women.

Methods: Mid-trimester amniotic fluid samples from healthy women with a singleton pregnancy were obtained at the time of genetic amniocenteses. SDF-1α concentrations were determined by enzyme-linked immunosorbent assay. Maternal and neonatal characteristics were recorded.

Results: A total of 210 samples were collected. According to the SDF-1α cutoff value established by the receiver operating characteristic curve analysis (< 6.42 *vs.* ≥ 6.42 pg/mL), there was a trend toward higher preterm birth rate, lower birth weight and lower 1-minute and 5-minute Apgar scores when SDF-1α levels increased ($p < 0.05$). The pair comparison between normal and selected pregnancy disorders (gestational diabetes, pre-eclampsia, and abnormal placentation) showed no statistical significance ($p > 0.05$). Pearson's correlations of SDF-1α to gestational age at delivery ($r = -0.151$) and birth weight ($r = -0.194$) were significant ($p < 0.05$). In the multivariate analysis on mid-trimester SDF-1α levels, maternal age at sampling (regression coefficient = –0.163) and 1-minute Apgar score (< 7 *vs.* ≥ 7, regression coefficient = 2.028) were both significant ($p < 0.05$).

Conclusion: Increased SDF-1α levels in mid-trimester amniotic fluid suggest a possible role in predicting pregnant women at risk of adverse neonatal outcomes including higher preterm birth rate, lower birth weight, and lower Apgar scores. [*J Chin Med Assoc* 2009;72(12):638–642]

Key Words: amniotic fluid, gestational diabetes, pre-eclampsia, pregnancy, stromal cell-derived factor 1

Introduction

Many cytokines are produced at the maternofetal interface (i.e. placenta, amnion and decidua) and considered to influence pregnancy outcomes.[1–3] Chemokines constitute a large family of structurally related cytokines and modulate many functions, such as enzyme secretion, cell adhesion, cytotoxicity, tumor cell growth, degranulation, and T-cell activation.[4,5]

The chemokine stromal cell-derived factor-1 (SDF-1) is expressed constitutively and ubiquitously in most tissues. It is also known as CXC chemokine ligand-12 (CXCL-12) or pre-B-cell growth-stimulating factor. SDF-1 is expressed in trophoblasts and decreases with gestational age.[6–8] It plays an important role in trophoblast cell proliferation or differentiation through the activation of ERK1/2 or antiapoptotic signaling pathways.[7] The interaction between CXCR4 and SDF-1 is involved in maternofetal immunological tolerance in all 3 trimesters of gestation and contributes to the invasion of extravillous trophoblasts when the spiral arteries are actively remodeling for establishment of uteroplacental circulation.[8] Furthermore, SDF-1 is a potent angiogenic stimulator of vascular endothelial growth factor expression, the main effector of neovascularization and the key inducer of vascular permeability.[9]

*Correspondence to: Dr Jenn-Jhy Tseng, Department of Obstetrics and Gynecology, Taichung Veterans General Hospital, 160, Section 3, Taichung-Kang Road, Taichung 407, Taiwan, R.O.C.
E-mail: t1018@vghtc.gov.tw • Received: July 17, 2009 • Accepted: November 16, 2009
†Jenn-Jhy Tseng and Ya-Fang Chen contributed equally to this study.

Several developmental defects, including B-cell lymphopoiesis, bone marrow myelopoiesis, congenital abnormality (e.g. ventricular septal defect) and even perinatal death, occurred in mice mutants lacking SDF-1.[6]

The constituents of mid-trimester amniotic fluid may reflect changes occurring in the maternofetal interface.[2] However, it is currently not known what variables affect SDF-1 levels or whether amniotic fluid SDF-1 levels correlate with pregnancy outcomes.

Two spliced forms of SDF-1 (SDF-1α and SDF-1β) have been identified, both of which have identical amino acid sequences except for the presence of 4 additional amino acids at the carboxyl terminus of SDF-1β.[10] The significance of the existence of the 2 spliced forms of SDF-1 is unclear. The different sensitivities of SDF-1α and SDF-1β to proteolytic processing provide a mechanism for chemokine functional regulation and reveal a functional difference between the 2 spliced forms. In this prospective study, we measured the concentration of SDF-1α in second trimester amniotic fluid samples and correlated this with pregnancy outcomes.

Methods

Patients

Mid-trimester amniotic fluid samples were obtained from pregnant women undergoing genetic amniocentesis at Taichung Veterans General Hospital from 2006 to 2008. All samples were collected from healthy women with a singleton pregnancy. The main indications for amniocentesis included: advanced maternal age, abnormal maternal serum screening result, and high parental anxiety levels. The pregnant women had no precedent risk factors for a high-risk pregnancy, such as a pre-existing medical or surgical illness, or obstetrics-related conditions. These pregnant women continued with regular antenatal visits. Fetal aneuploidies or major anomalies were excluded from the analysis. The clinical characteristics were recorded for further analysis. Pre-eclampsia was defined as blood pressure ≥ 140/90 mmHg after 20 weeks of gestation and proteinuria ≥ 300 mg/24 hours or ≥ 1 + dipstick (30 mg/dL).[11] According to the 100-g oral glucose tolerance test, diagnosis of gestational diabetes requires ≥ 2 abnormal values.[12] Abnormal placentation included placenta previa with or without placenta accreta (abnormal trophoblast invasion into the uterine myometrium). Small for gestational age describes a neonate whose birth weight is at least 2 standard deviations below the mean (≤ –2 SD) for the infant's gestational age.[13] This research protocol was approved by the Medical Research Council (Institutional Review Board) of Taichung Veterans General Hospital (941003/C05168), and informed consent was obtained from all patients.

Enzyme-linked immunosorbent assay (ELISA)

Amniotic fluid specimens were centrifuged at 1,000*g* for 10 minutes. The supernatants were collected and kept at –80°C until required. SDF-1α concentrations were determined with RayBio® Human SDF-1α ELISA kit (RayBiotech Inc., Norcross, GA, USA) according to the manufacturer's instructions. Briefly, supernatants of amniotic fluid and standards were pipetted into wells precoated with an antibody specific for human SDF-1α. After incubation overnight at 4°C and a total of 4 washes with wash buffer, biotinylated anti-human SDF-1α antibody, horseradish peroxidase-conjugated streptavidin and tetramethylbenzidine substrate solution were added sequentially into the wells. After the addition of stop solution, the optical density was determined using a microplate reader set to 450 nm. The intraassay and interassay coefficients of variation were less than 10% and 12%, respectively. Curve-fitting and dose interpolation were performed by software provided with the optical density reader. Samples were measured in triplicate. To exclude the effects of storage time on SDF-1α levels, the specimens from 2 different storage times for each of the 10 amniotic fluid samples were chosen and compared.

Statistical analysis

Statistical analysis was performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA) and MedCalc version 9.3 (MedCalc Software, Mariakerke, Belgium). A receiver operating characteristic curve was constructed to establish the cutoff values of SDF-1α with different variables. The area under the receiver operating characteristic curve and 95% confidence interval of the curve were analyzed. Predictive cutoffs were then determined. Based on the established cutoff point, continuous (maternal age at sampling, body mass index at sampling, gestational weeks at sampling, gestational weeks at delivery, and birth weight) and category variables (parity, preterm birth rate, delivery mode, birth weight category, Apgar scores at 1 minute and 5 minutes, and gender) were further analyzed using Fisher's exact test, Mann-Whitney U test, Pearson's χ^2 test, and Yates' correction of contingency as appropriate. Selected maternal follow-up outcomes including gestational diabetes, pre-eclampsia, and abnormal placentation were also investigated. Pearson's correlation coefficient was used to correlate SDF-1α concentration

and continuous variables. Multivariate regression analyses with backward selection were then conducted. For all analyses, $p<0.05$ was considered significant. The nonparametric Wilcoxon signed-rank test for 2 related samples was used for analyzing the effect of storage time on SDF-1α levels.

Results

A total of 210 mid-trimester amniotic fluid samples were collected. The storage time, which ranged from 2 to 4 months, did not affect SDF-1α concentration ($p>0.05$). The maternal and neonatal characteristics are shown in Table 1. We identified a cutoff value of SDF-1α (6.42 pg/mL), which would provide an optimal sensitivity and specificity in predicting Apgar score at 1 minute (<7 *vs.* ≥7) ($p=0.046$). There was a trend toward higher preterm birth rate ($p=0.048$), lower birth weight ($p=0.0396$), higher small for gestational age rate ($p=0.004$) and lower 1-minute and 5-minute Apgar scores ($p=0.0004$ and 0.039, respectively) when the SDF-1α level was ≥6.42 pg/mL.

As shown in Table 2, the overall *p* value between normal (158 cases) and selected pregnancy outcomes (7 gestational diabetes, 13 pre-eclampsia, and 5 abnormal

Table 1. Maternal and neonatal characteristics*

Characteristics	Mean ± SD or *n* (%)		Overall *p*
	<6.42 (*n*=180)	≥6.42 (*n*=30)	
Maternal age at sampling (yr)	34.50 ± 4.26	33.83 ± 4.23	0.171†
Parity (% nulliparity)	66 (36.7)	13 (43.3)	0.621‡
BMI at sampling (kg/m^2)	27.29 ± 3.66	26.47 ± 4.22	0.336†
Gestational weeks at sampling	17.08 ± 1.98	16.77 ± 1.14	0.656†
Gestational weeks at delivery	38.42 ± 2.72	36.83 ± 4.65	0.128†
Preterm birth rate (<37 wk)	22 (12.2)	8 (26.7)	0.048§
Delivery mode (% vaginal delivery)	99 (55.0)	16 (53.3)	1.000‡
Birth weight (g)	3,094.53 ± 599.56	2,643.80 ± 1,001.58	0.0396†
Birth weight category (% SGA)	2 (1.1)	4 (13.8)	0.004§
Apgar score at 1 min			0.0004§
<7	11 (6.1)	9 (30.0)	
≥7	169 (93.9)	21 (70.0)	
Apgar score at 5 min			0.039§
<7	6 (3.3)	4 (13.3)	
≥7	174 (96.7)	26 (86.7)	
Gender of neonate (% male)	96 (53.3)	17 (56.7)	0.888‡

**The cutoff point (6.42 pg/mL) was established by receiver operating characteristic curve analysis; †Mann-Whitney U test; ‡Yates' correction of contingency; §Fisher's exact test. SD = standard deviation; BMI = body mass index; SGA = small for gestational age.*

Table 2. Selected maternal follow-up outcomes*

Outcome	*n* (%)		Overall *p* value†	Pair comparison *p* value‡
	<6.42 (*n*=158)	≥6.42 (*n*=25)		
			0.233	
Normal§	139 (88.0)	19 (76.0)		–
GDM	6 (3.8)	1 (4.0)		1.000
Pre-eclampsia	10 (6.3)	3 (12.0)		0.379
Abnormal placentation‖	3 (1.9)	2 (8.0)		0.125

**The cutoff point (6.42 pg/mL) was established by receiver operating characteristic curve analysis; †Pearson's χ^2 test; ‡Fisher's exact test; §normal is the base set; ‖included placenta previa with or without placenta accreta. GDM = gestational diabetes mellitus.*

placentation) was not significant ($p = 0.233$). The pair comparison between normal and each of the selected pregnancy outcomes also showed no significant difference ($p > 0.05$).

In Table 3, the correlation of mid-trimester amniotic SDF-1α levels to continuous variables found that gestational age at delivery ($r = -0.151$, $p = 0.028$) and birth weight ($r = -0.194$, $p = 0.0048$) were statistically significant. This is consistent with the findings shown in Table 1. According to multivariate regression analysis on mid-trimester SDF-1α levels (Table 4), maternal age at sampling (regression coefficient = –0.163, $p = 0.0057$) and Apgar score at 1 minute (<7 *vs.* ≥7, regression coefficient = 2.028, $p = 0.019$) were significant variables.

Discussion

It has been suggested that SDF-1 is expressed in trophoblasts and decreases with gestation time. It is involved in maternal-fetal immunological tolerance in all trimesters of pregnancy.[8] SDF-1 is one of many factors involved in the process of trophoblast invasion, which is responsible for the establishment of uteroplacental blood flow after remodeling of the spiral arteries. It also suppresses apoptosis and enhances trophoblast cell survival during pregnancy.[14] Adjacent structures such as the placenta, fetal membranes and the decidua are thought to participate in the formation of amniotic fluid.[2] Therefore, we chose amniotic fluid as a safely available sample from which to measure the SDF-1α levels in order to obtain information on what is occurring at the maternofetal interface. This may be predictive of pregnancy outcomes.

The present study demonstrated that a higher SDF-1α level was predictive for a higher preterm birth rate, lower birth weight, higher small for gestational age rate, and lower Apgar scores. Our data suggested that increased SDF-1α levels in mid-trimester amniotic fluid are related to the occurrence of adverse pregnancy outcomes and possibly reflect the events taking place at the maternofetal interface in response to changes in uteroplacental function, uterine arterial remodeling, and circulating levels of other cytokines in early gestation. It is possible that a regulated suppression of the apoptotic cascade through a higher SDF-1α level is required for the maintenance of improved uteroplacental functions when the pregnancy has been primarily associated with functional impairment of trophoblast attachment, migration, or invasiveness affected by different intra- and extracellular effector molecules.

A strong predisposing factor for pre-eclampsia is defective trophoblast invasion.[15,16] In contrast, placenta previa increta/percreta, a severe form of abnormal placentation, is associated with excessive trophoblast invasion into the uterine myometrium during placental development.[17] Although SDF-1α is related to trophoblast invasion and survival, we did not find any significant differences in the concentrations of SDF-1α between normal pregnancies and those with subsequent pre-eclampsia or abnormal placentation. In the pre-eclampsia group (13 patients), the highest mid-trimester amniotic fluid SDF-1α level (15.84 pg/mL) came from a patient with HELLP syndrome (hemolytic anemia, elevated liver enzymes, and low platelets). Furthermore, in the abnormal placentation group (5 patients), the highest SDF-1α level (8.08 pg/mL) occurred in a patient with placenta increta (excessive trophoblast invasion of the uterine myometrium with

Table 3. Correlation of mid-trimester amniotic stromal cell-derived factor-1 (SDF-1α) levels (pg/mL) with continuous variables

Variable	Pearson's correlation coefficient	*p*
Maternal age at sampling	–0.122	0.079
BMI at sampling	–0.058	0.407
Gestational weeks at sampling	–0.088	0.202
Gestational weeks at delivery	–0.151	0.028
Birth weight	–0.194	0.0048

BMI = body mass index.

Table 4. Multivariate regression analysis on mid-trimester stromal cell-derived factor-1 (SDF-1α) levels (pg/mL)

Variable	Regression coefficient	β	Standard error	*t*	*p*
Constant	13.170	–	3.446	3.822	0.0002
Gestational weeks at sampling*	–0.256	–0.141	0.131	–1.956	0.0519
Maternal age at sampling*	–0.163	–0.202	0.058	–2.796	0.0057
Apgar score at 1 min†	2.028	0.163	0.857	2.365	0.019

*Note. This model includes the following variables: maternal age at sampling, parity, body mass index, gestational weeks at sampling, birth weight, birth weight category, and Apgar score at 1 minute and 5 minutes. All variables with a p value ≤ 0.10 are shown. Backward regression method was used. The model's entire p = 0.006 (analysis of variance F = 4.297). *Continuous; †< 7 compared with ≥ 7 (reference).*

abnormal neovascularization). More patients should be studied to determine the role of SDF-1α in the development of specific pregnancy disorders.

In addition to the 210 cases in the present study, 9 cases with chromosomal abnormalities were diagnosed (3 numerical, 3 structural and 3 sex chromosomal disorders). The SDF-1α levels of these cases were also measured during the study period. The relationship between the development of a chromosomal disorder with the SDF-1α level in the mid-trimester amniotic fluid was not significant ($p > 0.05$). However, 2 cases with trisomy 21 among the 9 cases with chromosomal disorders had the highest SDF-1α levels in the group (7.73 pg/mL and 8.99 pg/mL, respectively). One case (46,XY) with cystic hygroma diagnosed at 20 weeks of gestation had a SDF-1α level of 24.65 pg/mL at 15 weeks of gestation. It would be worthwhile studying the difference in SDF-1α between normal and abnormal pregnancies further.

In conclusion, increased SDF-1α levels in mid-trimester amniotic fluid suggest a possible role for predicting pregnant women at risk of a higher preterm birth rate, lower birth weight, and lower Apgar scores. However, the clinical usefulness of this test for pathological pregnancies needs to be investigated further.

Acknowledgments

We thank the Biostatistics Task Force, Taichung Veterans General Hospital, Taichung, Taiwan, for statistical assistance. This study was supported by research grants from Taichung Veterans General Hospital (TCVGH-956403C and TCVGH-986405C).

References

1. Hunt JS, Petroff MG, Burnett TG. Uterine leukocytes: key players in pregnancy. *Stem Cell Dev Biol* 2000;11:127–37.
2. Heikkinen J, Mottonen M, Pulkki K, Lassila O, Alanen A. Cytokine levels in midtrimester amniotic fluid in normal pregnancy and in the prediction of pre-eclampsia. *Scand J Immunol* 2001;53:310–4.
3. Zourbas S, Dubanchet S, Marta J, Chaouat G. Localization of pro-inflammatory (IL-12, IL-15) and anti-inflammatory (IL-11, IL-13) cytokines at the fetomaternal interface during murine pregnancy. *Clin Exp Immunol* 2000;126:519–28.
4. Rollins BJ. Chemokines. *Blood* 1997;90:909–28.
5. Murphy PM, Baggiolini M, Charo IF, Hébert CA, Horuk R, Matsushima K, Miller LH, et al. Internatalunion of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev* 2000;52:145–76.
6. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, Yoshida N, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* 1996;382:635–8.
7. Wu X, Li DJ, Yuan MM, Zhu Y, Wang MY. The expression of CXCR4/CXCL12 in first-trimester human trophoblast cells. *Biol Reprod* 2004;70:1877–85.
8. Yang Y, Zou L, Li M, Zhao Y. CXCL12/CXCR4 expression in trophoblasts takes part in materno-fetal immune tolerance and vascular remodeling. *J Huazhong Univ Sci Technolog Med Sci* 2006;26:466–8.
9. Brooks HL Jr, Caballero S Jr, Newell CK, Steinmetz RL, Watson D, Segal MS, Harrison JK, et al. Vitreous levels of vascular endothelial growth factor and stromal-derived factor 1 in patients with diabetic retinopathy and cystoid macular edema before and after intraocular injection of triamcinolone. *Arch Ophthalmol* 2004;122:1801–7.
10. De La Luz Sierra M, Yang F, Narazaki M, Salvucci O, Davis D, Yarchoan R, Zhang HH, et al. Differential processing of stromal-derived factor-1α and stromal-derived factor-1β explains functional diversity. *Blood* 2004;103:2452–9.
11. Cummingham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC, Wenstrom KD. Hypertensive disorders in pregnancy. In: Cummingham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC, Wenstrom KD, eds. *Williams Obstetrics,* 21st edition. New York: McGraw-Hill, 2005:760–808.
12. Cummingham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC, Wenstrom KD. Diabetes. In: Cummingham FG, Leveno KJ, Bloom SL, Hauth JC, Gilstrap LC, Wenstrom KD, eds. *Williams Obstetrics,* 21st edition. New York: McGraw-Hill, 2005:1169–88.
13. Lee PA, Chernausek SD, Hokken-Koelega ACS, Czernichow P. International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24–October 1, 2001. *Pediatrics* 2003;111:1253–61.
14. Jaleel MA, Tsai AC, Sarkar S, Freedman PV, Rubin LP. Stromal cell-derived factor-1 (SDF-1) signalling regulates human placental trophoblast cell survival. *Mol Hum Reprod* 2004;10:901–9.
15. Bjercke S. Normal implantation is important in order to avoid pregnancy complications and diseases later in life. *Tidsskr Nor Laegeforen* 1999;119:3903–8.
16. Guzin K, Tomruk S, Tuncay YA, Naki M, Sezginsoy S, Zemheri E, Yucel N, et al. The relation of increased uterine artery blood flow resistance and impaired trophoblast invasion in pre-eclamptic pregnancies. *Arch Gynecol Obstet* 2005;272:283–98.
17. Catanzarite VA, Stanco LM, Schrimmer DR, Conroy C. Managing placenta previa/accreta. *Contemp Obstet Gynecol* 1996;41:66–95.