LEVELS OF PFCs AND DEVELOPMENTAL EFFECTS IN INDUSTRIALIZED CITIES IN KOREA

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Introduction

Perfluorinated chemicals (PFCs) can be currently detected in various environmental media and humans[1,2]. Significant developmental effects of PFCs in humans have recently been reported. PFOS and PFOA concentrations in cord serums were inversely correlated with birth weight and size[3,4,5]. However, these developmental effects remain unclear and there were several reports demonstrating inconsistent association between PFCs levels and birth outcomes. Further studies with more diverse populations and geographical locations are required to draw a solid causal effect relationship.

In an attempt to examine the relationship between developmental effects and level of exposure, we investigated human serum samples obtained from the Korean general population. Because levels of PFCs in human serum are associated with geographic factors and types of industry, we included two different types of industrial cities in Korea. Daegu, the third largest city, has previously shown the highest levels of PFOAs ever reported. Her major industries include textile, rubber and plastic manufacturers, which are generally considered as industrial sources of PFC exposure [6,7]. Pohang is a port city heavily concentrated on iron and steel manufacturing, which is not directly related with source of PFC-generating industry. Perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexane sulfonate (PFHxS) among PFCs are the most prominent perfluorochemicals in human samples[12].

The present study analyzed levels of these three PFCs in umbilical cord blood and maternal blood samples at delivery. The PFCs levels were statistically analyzed to determine association with demographic and physical conditions of participating subjects such as gestational age, parity, head circumference, birth weight and length, etc. Transplacental transfer efficiency (TTE) of each PFC was also determined for individual maternal/cord sera matching samples. Korea, one of fastest industrializing countries in the world, has shown a trend of increase on PFCs levels in historical samples [1]. Thus, we look into the levels of PFC exposure and developmental effects in these cities.

Methods

Study subjects and sample collection

The maternal and UCB serum samples were collected from seventy volunteered pregnant women who visited the regional hospitals for delivery in Daegu and Pohang, South Korea, from March through August in 2011. Demographic data of mothers and infants such as birth weight, length, ponderal index, age, parity and body mass index (BMI) were obtained from the chart reviews and questionnaires.

Five ml of maternal or umbilical cord blood at delivery was collected into 10 ml red-topped vacutainers immediately after delivery and the collected whole blood was centrifuged at 1500 rpm for 20 min. The serum was separated and kept at -70^{o}C until chemical analysis. The study protocol was approved by the Catholic University of Daegu Medical Center Research Ethics Board.

Sample extraction and analysis

The serum samples were extracted analyzed with ion-pairing and LC/MS/MS (Thermo Electron Corporation, USA) under quality control described previously [8].

Statistical analysis

Data were expressed as the mean ± standard deviation and median for continuous variables and the frequency and percentage for categorical variables, respectively. Student's *t*-tests were used to assess the effects of birth outcomes on PFC concentrations in maternal and UCB samples. To further determine the effects of birth outcomes, multiple logistic regression analysis was performed after adjusting for confounders such as maternal age and gestational age. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated for each parameter of birth outcomes. The relationship of PFC levels between maternal and UCB samples were determined by Pearson's correlation analysis. Statistical significance was set with a two-sided significance level of 0.05. All statistical analyses were performed by SPSS for windows 16.0.

Results and discussion

Study population

Among seventy samples of maternal and umbilical cord sera, fifty nine samples were mother-infant pairs and eleven samples were not. The statistical analysis for birth outcomes and placental transfer efficiency was performed for fifty nine paired samples only. None of the mothers had a history of alcohol drinking or smoking prior to or during the pregnancy. All the deliveries were singleton births without congenital malformations.

Levels of PFOS, PFOA and PFHxS in maternal and umbilical cord sera

PFOS, PFOA and PFHxS were detected in all the maternal and UCB serum samples analyzed. The order of mean concentrations in both maternal and UCB samples (highest to lowest) was PFOS>PFOA>PFHxS. Levels of UCB samples were lower than those of maternal samples. The maternal level of PFOS in this study (10.77ng ml^{-1}) was higher than domestic cities in Korea [1,9], but lower than Chinese [10], Canadian [11] and Danish studies [4]. The maternal level of PFOA (2.73ng ml^{-1}) was also higher than domestic city, but lower than Danish study. Level of maternal PFHxS (1.35ng ml^{-1}) was similar to domestic city but much lower than Canadian study. PFC levels of pregnant women in this study were generally lower than those in western countries, but the levels in heavily industrialized cities such as Daegu and Pohang were higher than Korean metropolitan city like Seoul. Our results suggest that level of industrialization may affect the exposure level of the general population.

Transplacental transfer efficiencies (TTEs) were estimated for the 59 maternal/UCB sera pairs by dividing the PFC concentrations in UCB serum by maternal serum concentrations at delivery. The order of TTE value was PFOA(0.84) > PFHxS(0.57) > PFOS(0.35). Levels of PFCs in UCB serum were highly correlated with those of respective PFCs in maternal serum (PFOS, p=0.0001; PFOA, p=0.0007; PFHxS, p=0.0001). The study indicates that these chemicals cross the placental barrier during pregnancy and result in exposure of developing fetus. Our TTE results are consistent with several reports, which reported the relationship between chain length and the functional group of PFC and the TTE values [9,12]. The composition of PFCs in maternal samples (PFOS, 72%; PFOA, 18%; PFHxS, 10%) was altered in UCB samples (PFOS, 55%; PFOA, 33%; PFHxS, 12%). The results suggest that difference of TTEs may lead to the altered distributions of PFCs in UCB samples, which substantially increased PFOA and decreased PFOS portions.

Effects on birth outcomes

Student's *t*- test revealed significant differences of birth weight, birth length and ponderal index between birth outcome and high maternal PFOA levels among infants below median levels. Levels of PFCs in UCB serum were not significantly related with birth outcomes (Table 1). Among PFCs analyzed, maternal PFOA level only was significantly associated with a parity > 1.

To estimate odds ratios (ORs) and 95% confidence intervals (CIs) for birth outcomes, multiple logistic regression models were used in this study. Multiple regression analysis also showed correlation between parity and maternal PFOA levels (Table 2). Maternal PFOS levels showed a marginal inverse association with ponderal index using a crude (i.e., no adjustments) model. This inverse association disappeared after adjusting for gestational age, but it became significant again after adjusting for both gestational age and maternal age (OR= 0.22; 95%CI, 0.05-0.90). Maternal PFHxS was marginally associated with decrease of birth length by the crude model, but this effect disappeared after adjustment. UCB PFHxS showed a significant inverse association with birth weight (OR=0.26; 95% CI, 0.08-0.85) and a marginal inverse association with birth length (OR=0.33; 95% CI, 0.09-1.16) at both crude and fully adjusted models (Table 2).

The present study demonstrated that maternal PFOA level significantly affected the birth outcomes including birth weight, birth length and ponderal index, as analyzed with Student's *t*-test. Further analysis with multiple logistic regression models after adjusting for confounders demonstrated inverse associations of maternal PFOS and UBC PFHxS levels with ponderal index and birth weight, respectively. This is the first report demonstrating a significant inverse association of umbilical cord PFHxS with birth weight.

Two city comparison

While all three PFCs levels from pregnant women in Daegu were higher than those of Pohang, umbilical cord blood did not show such a geographical difference. Comparison of the two cities showed that a city of PFC-use industry has significantly higher levels of all three PFCs than the other in maternal samples, suggesting that the PFC-related industries may affect the levels of the general population that is not directly exposed to the industrial environment. While the pathways of PFC exposure are too diverse to pinpoint, our results may add additional evidence that industry of PFC-use could be a critical PFC source of origin. In a previous study in 2004, Daegu showed a highest level of PFOA ever measured in a general population as a single case (256ng ml^{-1}) and its average level of PFOA (88ng ml^{-1}) was also higher than any other cities in the world [6,7]. The present study showed that levels collected in 2011 (3.22ng ml^{-1}) was a lot lower than in 2004, although population in this study includes only young pregnant women. Such decrease may be due to the effort of municipal authority and the phase out of the related compounds in the market. However, the PFC level of Daegu is still higher than other major Korean domestic cities. It is speculated that presence of high PFC-use industry may be related with higher level of the general population in this region.

Taken together, this study further confirms prenatal exposure of PFC that results in exposure of developing fetus and suggests the associations of birth outcomes with specific PFC exposure. In addition, the type of industry may play an important role in affecting the level of the general population where the relevant industry operates.

Our results may lead to a larger scale investigation into developmental effects of PFCs in the future and contribute to understanding sources of exposure from a variety of populations in the globe.

Acknowledgement

This work was supported by POSCO, South Korea. Authors appreciate great helps from Drs. SY Hong, MS Koh and CD Han.

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Table 1. Maternal (m-) and umbilical cord (c-) serum PFOS, PFOA, and PFHxS concentrations (mean ± SD) by infant characteristics (paired samples
of maternal and umbilical cord sera n=59)

Characteristics	No.	m-PFOS	m-PFOA	m-PFHxS	c-PFOS	c-PFOA	c-PFHxS
Parity							
0	27	11.10 ± 7.32	2.86 ± 0.95	1.35 ± 0.64	3.33 ± 1.85	2.19 ± 0.65	0.68 ± 0.31
≥ 1	32	9.77 ± 3.20	2.40 ± 0.59	1.18 ± 0.47	3.37 ± 1.33	2.01 ± 0.84	0.66 ± 0.35
p-value*		0.39	***0.03***	0.24	0.92	0.38	0.87
Birth weight (kg)							
< 3.19 (median)	28	11.28 ± 6.65	2.83 ± 0.88	1.33 ± 0.50	3.36 ± 1.63	2.12 ± 0.55	0.67 ± 0.25
≥ 3.19	31	9.56 ± 4.08	2.41 ± 0.68	1.20 ± 0.60	3.35 ± 1.56	2.07 ± 0.92	0.67 ± 0.39
p-value*		0.24	***0.05***	0.38	0.98	0.78	0.92
Birth length (cm)							
< 49.0 (median)	19	11.89 ± 7.25	2.91 ± 0.95	1.35 ± 0.48	3.48 ± 1.93	2.09 ± 0.51	0.65 ± 0.21
≥ 49.0	40	9.66 ± 4.32	2.47 ± 0.69	1.22 ± 0.59	3.29 ± 1.41	2.10 ± 0.86	0.68 ± 0.38
p-value*		0.23	***0.04***	0.42	0.68	0.99	0.75
Ponderal index (g/cm^3)							
< 2.61 (median)	28	11.45 ± 5.05	2.83 ± 0.87	1.41 ± 0.61	3.36 ± 1.28	2.05 ± 0.58	0.69 ± 0.36
≥ 2.61	31	9.41 ± 5.74	2.41 ± 0.69	1.13 ± 0.47	3.35 ± 1.83	2.14 ± 0.90	0.65 ± 0.31
p-value*		0.15	***0.04***	***0.05***	0.98	0.67	0.66

* Calculated by Student's *t*-tests

Table 2. Odds ratios and 95% confidence intervals of maternal (m-) and unbilical cord (c-) serum PFOS, PFOA, and PFHxS (≥ median versus < median [reference])
by infant characteristics*(paired samples o fmaternal and umbilical cord sera, n=59)

Dependent variables	m-PFOS		m-PFOA		m-PFHxS		c-PFOS		c-PFOA		c-PFHxS	
Birth weight												
Crude	1.07	(0.38-2.97)	0.47	(0.17-1.32)	0.53	(0.19-1.50)	0.81	(0.29-2.26)	0.47	(0.17-1.32)	**0.30**	**(0.10-0.88)**
Adjusted for GA	1.22	(0.42-3.52)	0.47	(0.17-1.35)	0.58	(0.20-1.68)	0.89	(0.31-2.51)	0.47	(0.17-1.36)	**0.29**	**(0.10-0.87)**
Fully adjusted‡	0.98	(0.32-3.03)	0.54	(0.17-3.03)	0.57	(0.19-1.75)	0.85	(0.28-2.57)	0.51	(0.17-1.59)	**0.26**	**(0.08-0.85)**
Birth length												
Crude	0.90	(0.30-2.69)	0.48	(0.16-1.47)	**0.38**	**(0.12-1.19)**	0.66	(0.22-1.98)	0.48	(0.16-1.47)	**0.38**	**(0.12-1.19)**
Adjusted for GA	1.18	(0.37-3.82)	0.48	(0.15-1.54)	0.46	(0.14-1.53)	0.78	(0.24-2.47)	0.47	(0.15-1.54)	**0.35**	**(0.10-1.18)**
Fully adjusted‡	0.97	(0.29-3.27)	0.44	(0.12-1.58)	0.44	(0.13-1.50)	0.78	(0.24-2.56)	0.45	(0.13-1.58)	**0.33**	**(0.09-1.17)**
Ponderal index												
Crude	**0.35**	**(0.12-1.01)**	0.47	(0.17-1.32)	0.53	(0.19-1.50)	0.47	(0.17-1.32)	0.62	(0.22-1.73)	0.53	(0.19-1.50)
Adjusted for GA	0.43	(0.14-1.36)	0.43	(0.14-1.36)	0.69	(0.22-2.13)	0.69	(0.17-1.63)	0.63	(0.20-1.93)	0.46	(0.15-1.47)
Fully adjusted‡	**0.22**	**(0.05-0.90)**	0.56	(0.16-2.01)	0.64	(0.19-2.23)	0.64	(0.10-1.37)	0.84	(0.25-2.88)	0.46	(0.13-1.62)

Abbreviations: GA, gestational age

* Dependent variables were categorized as follows: for parity, ≥ 1 versus 0 [reference]; for the other variables, ≥ median versus < median [reference]

† Calculated by multiple logistic regression models controlling for gestational age and maternal age

‡ Calculated by multiple logistic regression models controlling for gestational age, maternal age, BMI and parity