論文内容の要旨

論文題目：**The Cord Blood Levels of Heavy Metals and Trace Elements and Their Relationship with Neurodevelopment of Infants of Chitwan, Nepal: A Follow- up from birth to 6 months of Age**

和訳：ネパール・チトワン郡の乳児における臍帯血中重金属・微量元素濃度と神経発達の関連：出生から生後6カ月までのフォローアップ調査

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Summary

1. INTRODUCTION

Enormous efforts have been devoted to clarify determinants of neurodevelopment of humans. During the prenatal period, exposure of fetuses to heavy metals [lead (Pb), arsenic (As) and cadmium (Cd)] or deficiency of essential trace elements [zinc (Zn), selenium (Se) and copper (Cu)] were reported to be associated with prenatal and postnatal neurodevelopment. Maternal psychological conditions and *in utero* growth environment also affected the neurodevelopment during prenatal and postnatal period. During the postnatal period, growth environments (e.g., presence of appropriate toys, siblings, attitude of parents) are the prominent determinants of neurodevelopment.

The present study was conducted in Chitwan valley of lowland (Terai) Nepal. The author targeted this area because he suspected that the local ecosystem of the area was contaminated with Pb, Cd and As. Since Chitwan is located in the junction where a highway from Kathmandu connects to east-west highway, numerous vehicles are supposed to emit lead to the environment. In addition, for the Chitwan people, rice is the staple foods as well as the source of Cd and Pb, and the area is reported to be one of the “hot spots” of As contamination. The Zn deficiency reported to be the health problem in lowland Nepal.

Numbers of studies have evaluated the effect of cord blood lead on later neurodevelopment, but most of the studies evaluate such effect in developed countries. The dose-response relationship and time lag effect remains inconclusive for lead. Limited number of studies evaluated the effect of arsenic, cadmium, and copper on neurodevelopment in humans and the effect of deficiency of trace elements (Zn, Se and Cu) on later neurodevelopment (from birth to 6 months). The objectives of the present study were (1) to evaluate the *in utero* exposure to heavy metals (Pb, As and Cd) and trace elements (Zn, Se and Cu), pregnancy maternal conditions, and postnatal growth environments (analyzed as explanatory variables), (2) to evaluate the neurodevelopmental indicators of babies at birth [i.e., Brazelton neonatal behavioural assessment scale, third edition (NBAS III)] and at 6 months from birth (i.e., Bayley scale of infant development, second edition (BSID II) (analyzed as response variables), and (3) to investigate the association between *in utero* heavy metals and trace elements exposures, prenatal maternal conditions or postnatal growth environment, and neurodevelopmental indicators after birth.

2. METHODS

The data analyzed in the present study were collected in May 2008, September to October 2008, and March 2009. After the pilot study in May 2008, one hundred pregnant mothers were recruited in September to October 2008, and mother-newborn pairs were investigated just after the delivery. Mother-infant pair were again investigated after 6 months (n=98) in March 2009. Neurodevelopmental indicators were evaluated at birth (by NBAS III scores, and at six months from birth (by BSID II scale). Heavy metals, and trace elements in cord blood (n=94) were analyzed. The information for maternal psychological conditions and *in utero* growth environment, and postnatal growth environments were also evaluated using Rosenberg self esteem scale (RSES), birth weight and home observation of measurement of environment scale (HOME scale), respectively . Confounding variables were controlled. Cord blood samples, transported to the laboratory in Japan in October 2008, were analyzed by using ICP-MS in May 2009. Univariate analyses were conducted to examine the associations between explanatory variables (heavy metals and trace elements in cord blood, pregnancy maternal conditions, and postnatal growth environments) or confounding variables and response variables (NBAS III cluster scores at birth and BSID II cluster scores at 6 months after birth). Explanatory variables or confounding variables that were associated with response variables at p-value less than 0.1 were forcedly entered to multivariate model.

The study protocol was approved by the ethical committee of the Graduate School of Medicine, the University of Tokyo (No. 2244) and that of Bharatpur general hospital, Chitwan Nepal.

3. RESULTS

In multivariate regression model, neurodevelopment indicators *at birth* (those evaluated by seven cluster scores of NBAS) was significantly associated with the following explanatory variables: Rosenberg self esteem score (one of the indicators of prenatal maternal psychological conditions) was inversely associated (p=0.03) with habituation cluster score, while frequency of massage during pregnancy (one of the indicators of prenatal maternal psychological conditions) and incumbent length of babies (one of the indicators of nutritional status of babies at birth) was positively associated (p=0.04 and p=0.01, respectively) with the score. Cord blood concentrations of Se and As were inversely associated with state organization cluster score (p=0.03) and state regulation cluster score (p=0.01), respectively. Birth weight (one of the indicators of nutritional status of babies at birth) was positively associated (p<0.01) with state organization cluster (Table 1).

Table 1. Multiple regression analysis of each cluster of NBAS III at birth [1] (n=94)

Response variables (cluster score)	Habituation	Orientation	State organisation	State regulation	Autonomic stability	Number of abnormal reflex
			[2]β (95% CI[3])			
Explanatory variables						
Heavy metals in cord blood						
Pb						
As				**-0.22 (-0.42−-0.02)**		
Cd					-0.16 (-0.35−0.04)	-0.18 (-0.38−0.02)
Trace elements in cord blood						
Zn						
Se			**-0.22 (-0.41−-0.02)**			
Cu						
Maternal psychological conditions						
Rosenberg self esteem score	**-0.21 (-0.41−-0.02)**					
Satisfaction with life scale score						
Frequency of massage during pregnancy	**-0.21 (-0.40−-0.01)**					
Indicators of nutritional status of newborns						
Body weight (g)		0.04 (-0.21−0.29)	**0.33 (0.08−0.51)**			
Incumbent length (cm)	**0.25 (0.06−0.45)**	-0.16 (-0.40−0.08)				
Head circumference (cm)		-0.20 (-0.43−0.04)				
Ponderal index (kg/m^3)			0.08 (-0.16−0.31)			
Model summary						
Adjusted R^2	0.11	0.15	0.19	0.11	0.08	0.04

[1]: Univariate analysis were conducted to investigate the association between explanatory variables and response variables and between confounding variables and response variables: see text (section 1.2, 1.3, 1.6, 1.7, 1.8, 1.9 and 1.10) for the definition of response, explanatory and confounding variables in the present study.
[2]β: Standardized coefficients (beta coefficient). CI[3:] Confidence interval
Only the results of multivariate models are shown. Explanatory variables that were associated with response variables in univariate analysis, at $p<0.1$ were entered to multivariate model (the variables shown in the table). Confounding variables that were associated with response variables at $p<0.1$ were also entered to multivariate model for adjustment (not shown). The confounding variables entered to each model were: education level of spouse in years and automobile possession (for orientation cluster score); Apgar score at 1 minute, education level of spouse in years and fish consumption frequency during pregnancy (for state organisation cluster score); Apgar score at 5 minutes, age of mother in years and parity (for state regulation cluster score), and mother's length of education in years (for autonomic stability cluster score and number of abnormal reflex cluster score).

Neurodevelopment indicators *at six months* from birth (those evaluated by three scores of BSID II) were significantly associated with the following explanatory variables: PDI score was positively associated (p=0.04) with HOME scale score (one of the indicators of postnatal growth environment), while BRS score was positively associated (p=0.03) with weight at birth (Table 2).

Table 2. Multiple regression analysis of each cluster of BSID II at 6 months after birth [1] (n=94)

Response variables	MDI	PDI	BRS
Explanatory variables		[2]β (95% CI[3])	
Heavy metals in cord blood			
Pb			
As			
Cd			
Trace elements in cord blood			
Zn	0.18 (-0.02−0.37)		
Se			
Cu			
Maternal psychological conditions			
Rosenberg self esteem score			
Satisfaction with life scale score			
Frequency of massage during pregnancy		-0.15 (-0.35−0.05)	
Indicators of nutritional status of newborns			
Body weight (g)			**0.23 (0.03−0.44)**
Incumbent length (cm)			
Head circumference (cm)			
Ponderal index (kg/m^3)		0.11 (-0.10−0.32)	
Indicator of nutritional status of infants at 6 months			
Body weight (g)			0.08 (-0.12−0.29)
Incumbent length (cm)			
Ponderal Index (kg/m^3)	0.16 (-0.05−0.36)		
Postnatal growth environment			
HOME scale score		**0.27 (0.01−0.53)**	0.19 (-0.03−0.42)
Household crowding			
Number of siblings			
Model summary			
Adjusted R^2	0.13	0.13	0.17

[1] Univariate analysis were conducted to investigate the association between explanatory variables and response variables and between confounding variables and response variables: see text (section 1.2, 1.3, 1.6, 1.7, 1.8, 1.9 and 1.10) for the definition of response, explanatory and confounding variables in the present study.
[2]β: Standardized coefficients (beta coefficient).
[3]CI: Confidence interval
Only the results of multivariate models are shown. Explanatory variables that were associated with response variables in univariate analysis, at p<0.1, were entered to multivariate model (the variables shown in the table). Confounding variables that were associated with response variables at p<0.1 were also entered to multivariate model for adjustment (not shown). The confounding variables entered to each model were: corrected age at BSID II assessment in days [for mental development index (MDI) score]; corrected age at BSID II assessment in days, education level of father in years and gestational age in days for [psychomotor development index (PDI) score]; parity, gestational age in days, and automobile possession [for behavioural rating scale (BRS) score].

4. DISCUSSION

The neurodevelopment status (evaluated by NBAS III and BSID II) in the present study was similar to that reported in the previous studies, observed in "general" populations which do not have apparent risk factors (as defined by authors) for neurodevelopmental deficit.

Despite many papers reported the inverse associations between *in utero* Pb exposure and infants neurodevelopment indicators, the present study did not detect such association as reported in preceding studies. It is thought that the association between cord blood Pb concentration and neurodevelopmental indicators was not found due to low cord blood Pb.

Only *in utero* exposure of As grounds for the neurodevelopmental harm among the heavy metals examined in the present study population. The problem of As contamination in drinking water is worldwide (WHO, 2001). Recent studies suggested that As affects the central nervous system (Wright et al., 2006; von Ehrenstein et al., 2007; Calderon et al., 2001; Rocha-Amador et al., 2007; Wasserman et al., 2004). This study is the first to report the adverse effect of prenatal As exposure on neurodevelopmental indicator at birth in relatively low exposure level.

Regarding trace elements, Zn deficiency as reported by Andersen (2007) and Christian et al. (2006) from the region, was not confirmed in the present study population.

Cord blood concentration of Se was inversely associated with neurodevelopment indicators at birth but not at 6 months. Although endemic Se intoxication cases have been reported in China, the exposure among the participants in the present level was estimated to be much lower. The reason for the inverse association between Se and neurodevelopment stay unclear.

The present study confirmed the positive association between birth weight and neurodevelopment after 6 months as observed by Tofail et al. (2009), Torres-Sanchez et al. (2007) and Wu et al. (2008) among healthy, full term children.

Similarly, the present study demonstrated the positive association between neurodevelopmental indicator at 6 months and home environment as observed by Torres-Sanchez et al. (2007) in comparable home environment in Mexico cohort.

5. CONCLUSION

In a hospital-based cohort of infants and mothers in Terai region, lowland Nepal, relationship between the neurodevelopment of the infants at birth as well as at 6 months after birth and potential determinants of the development including metals exposure, essential trace elements, nutritional status at birth, and postnatal growth environment was examined. The cord blood As concentration was significantly associated with a part of scales of neurodevelopment of the newborns. It suggests that high As exposure during prenatal period in babies may induce retardation of *in utero* neurodevelopment. *In utero* growth environment was found to be associated with neurodevelopment at birth and after 6 months, while the postnatal growth environment (home environment) is a determinant of neurodevelopment at 6 months after birth.