[Translated by Bin Li and published with the concurrence of the Chinese Journal of Endemiology 2004 Sep;23(5):463-5.]

EFFECTS OF HIGH FLUORIDE LEVEL ON NEONATAL NEUROBEHAVIORAL DEVELOPMENT

Jing Li,[ab] Li Yao,[ac] Qing-Liang Shao,[ac] Chun-Yan Wu[ab]

Daqing, China

ABSTRACT: The effects of excessive fluoride intake during pregnancy on neonatal neurobehavioral development and the neurodevelopment toxicity of fluoride were evaluated. Ninety-one normal neonates delivered at the department of obstetrics and gynecology in five hospitals of Zhaozhou County, Heilongjiang Province, China were randomly selected from December 2002 to January 2003. The subjects were divided into two groups (high fluoride and control) based on the fluoride content in the drinking water of the pregnant women. The results showed that the urinary fluoride levels of mothers from the high fluoride group were higher than those of the control group. There were significant differences in the neonatal behavioral neurological assessment score and neonatal behavioral score between the subjects in the endemic fluoride areas and the control group. There were also significant differences in the non-biological visual orientation reaction and biological visual and auditory orientation reaction between the two groups. It is concluded that fluoride is toxic to neurodevelopment and that excessive fluoride intake during pregnancy can cause adverse effects on neonatal neurobehavioral development.

Keywords: Fetal fluoride toxicity; High fluoride water; Neonate testing; Neurobehavioral development; Pregnancy and fluoride.

INTRODUCTION

Fluoride exists widely in human habitats. Long-term exposure to fluoride is regarded by the World Health Organization as both beneficial (preventing cavities and treating osteoporosis) and also recognized as harmful (causing mottled enamel and fluorosis of the bone) to human health.[1] Long-term intake of excess fluoride will result in toxic injury of overall organs with bone as the primary target organ.[2] Modern research indicates that fluoride can cross the placental barrier and enter the fetus and thereby accumulate in brain tissues, causing toxic impacts on the development and differentiation of brain neurocytes. The present work studied the impact of the high levels of fluoride in the living environment of expectant mothers on the neurobehavioral development of newborns by analyzing the neurobehavioral scores of neonates from regions with high fluoride water and normal fluoride water.

MATERIALS AND METHODS

Subjects and groups: Zhaozhou county of Heilongjiang Province is one of the regions of China with high fluoride in the drinking water. During the period between December 2002 and January 2003, 273 neonates were born at the local Women and Children Hospital, People’s Hospital of Zhaozhou County, Hospital of Traditional Chinese Medicine, Wan Bao Hospital, and Shuang Fa Hospital.

[a]Department of Pediatrics, Daqing Oil Felid General Hospital, Daqing 163001, China. Authors’ Working Units: [b]163001 Pediatrics Department, General Hospital of Daqing Oil Field (Li Jing and Wu Chunyan); [c]Pediatrics Department, Second Hospital Affiliated with Harbin Medical University (Yao Li and Shao Qingliang).

Ninety-one full-term, normal neonates were randomly selected for observation. Among the 91 neonates, 46 were male and 45 female. They were all delivered by natural delivery or surgical Caesarean birth. The mothers were all healthy women, with ages ranging from 20 to 31 without pregnant high blood pressure syndrome, heart disease, diabetes, or any infectious diseases, threatened abortion or family genetic disease and had not left their place of abode since pregnancy. The mothers and the neonates were divided into a high fluoride group and a normal control group based on the fluoride level in the drinking water of the place of residence. There were 44 mothers and babies in the high fluoride group from Shuanfa Village, Yongsheng Village, Yushu Village, Shuanglong Village, and Tuogu Village of Zhaozhou County. The fluoride level in the drinking water in these villages is 1.7–6.0 mg/L and without modification of the drinking water from fluoride-controlled wells it is 1.0 mg/L higher in fluoride content than the drinking water standard or the drinking water from discarded fluoride-controlled wells. There were 47 mothers and babies in the control group from Chapeng Village, Yongle Village, Wanbao Village, and Leyuan Village of the same county with a fluoride level in the drinking water of 0.5–1.0 mg/L.

Collection of Samples and Measurements: A urine sample was collected after the pregnant women were hospitalized but before labor began and stored under low temperature conditions. Urine samples were all sent to Heilongjiang Provincial Endemic Control Center of Harbin Medical University for measurement. The fluoride level was determined by fluoride ion selective electrode, and the experimental procedure strictly followed the internal controls of the laboratory.

Behavioral Neurological Assessment: The standard neonatal behavioral neurological assessment (NBNA) method was used for measurements, which were carried out by professionals (pediatric department physicians working in neonatal section trained in national NBNA training program and who passed the training by exams.) The examinations were carried out 1 to 3 days (24–72 hr) after delivery between two feedings at a room temperature of 24–28ºC in a half dark and quiet environment. Twenty items were included in the examination, such as neonatal behavioral capability, passive muscle tension, agonistic muscle tension, primary reflection, and general reactions. For each examined item, there are three rankings (0, 1, and 2): 0 for failure to elicit reactions or obvious abnormality, 1 for mild abnormality, and 2 for perfect normal reactions.

Statistical Treatment: The data are shown as mean±SD, and a *t* test was carried out between two samples.

RESULTS

The two study groups are located in the same area with similar climate, living habits, economic and nutritional conditions, and cultural backgrounds. There is no industrial fluoride contamination in the endemic fluoride or the non-endemic control area. As can be seen in Table 1, there was no statistically significant difference in the delivery mode, birth weight, infant length, and sex.

Table 1. General conditions of neonates from high fluoride group and control group

Group	Number of neonates	Male	Female	Caesarean birth	Natural delivery	Age of fetus (weeks)	Weight (kg)	Length (cm)
High fluoride	44	23	21	18	26	39.1±1.9	3.18±0.35	50.13±1.23
Control	47	23	24	19	28	39.6±1.6	3.13±0.52	50.14±1.17

Fluoride measurement results in urine samples: In the high fluoride group the urinary fluoride level averaged 3.58±1.47 mg/L, significantly higher than the normal range of 0.18–2.6 mg/L and was significantly different from the fluoride level in urine samples from the control group. When compared with the fluoride result in urine samples from the control group (1.74±0.96 mg/L), the difference is statistically very significant ($p<0.01$).

Comparison of neonatal neurobehavioural development: As seen in Table 2, neonatal neurobehavioural assessment of the neonates from the high fluoride group and the control group indicated that the neurobehavioural capability and agonistic muscle tension from the high fluoride group were impaired, resulting in a statistically significant lower overall (total) assessment score than in the control group ($p<0.05$).

Table 2. Neurobehavioral assessment scores of neonates from high fluoride group and control group (mean±SD)

Group	Number of neonates	Total NBNA score	Behavioral capability	Passive muscle tension	Agonistic muscle tension	Primary reflection	General reaction
High fluoride	44	36.48±1.09*	10.05±0.94*	7.89±0.32	6.80±0.70*	5.89±0.32	5.84±0.37
Control	47	38.28±1.10*	11.34±0.56*	7.87±0.34	7.40±0.68*	5.89±0.31	5.79±0.41

*Statistically significant.

Comparison of neurobehavioral capability of neonates: Table 3 shows that the various neurobehavioral capabilities, such as non-biological visual, biological visual, and auditory directional reactions of the neonates from the high fluoride group lagged behind those of the control group with differences that are statistically significant ($p<0.05$).

Table 3. Scores of various neurobehavioral capabilities of neonates (mean±SD)

Group	Number of neonates	Total score of behavioral capability	Tolerance to light	Tolerance to clicking sounds	Directional reaction to non-biological audition	Directional reaction to non-biological vision	Directional reaction to biological vision and audition	Placebo
High fluoride	44	10.05±0.94*	1.95±0.21	1.93±0.26	1.95±0.21	0.98±0.63*	1.09±0.64*	1.99±0.17
Control	47	11.34±0.56*	1.98±0.15	1.89±0.31	1.98±0.15	1.66±0.52*	1.77±0.48*	1.98±0.15

*Statistically significant.

DISCUSSION

Poisoning from fluoride in drinking water is the most common type of endemic fluoride disease. In endemic fluoride regions, the urine fluoride level exhibits a strong correlation with the fluoride concentration in the drinking water and with the intake of fluoride, and it is a specific indicator of the body fluoride intake and load level. The results of this study show that the urinary fluoride levels of expectant mothers in high fluoride areas are significantly increased, suggesting that in these areas there are higher accumulations of fluoride in the bodies of these mothers.

The subjects of this study were neonates, and NBNA indicators were used to evaluate the impact of high fluoride on the neurobehavioural development in them. NBNA is a credible and reliable method for the clinical examination of neonates. It is easy to conduct, economical, practical, and effective for assessing the neurobehavioral capability, agonistic muscle and passive muscle tensions, primary reflection and general reaction as high risk factors during the perinatal period.

Modern studies have confirmed that fluoride can cross the placenta and enter the body of the fetus and can cross the blood-brain barrier and accumulate in brain tissues. Fluoride that has crossed the blood-brain barrier and entered into brain tissues will damage the brain neural cells at different levels resulting in the abnormality of the development and differentiation of brain neural cells. The developing brain neural cells are quite sensitive to fluoride toxicity. High fluoride can result in damage to brain cells. However, because the damage is usually mild, obvious functional changes will not necessarily be readily observable. NBNA examination can help to detect mild damage to brain functions. The results of the examination indicate that high fluoride levels can cause adverse effects in the neurobehavioral development of neonates.Table 3 shows that the development of directional reflection of vision and audition of the neonates in the high fluoride group was adversely affected. The arch of the directional reflection of non-biological vision and biological vision and audition is relatively advanced and complicated and requires the participation and coordination of the integrated function of vision, audition, and perception. Therefore, the visual and aural orientation of the neonates is completed through the cerebral cortex and complex neural pathways.[4] The impairment or loss of these functions indicates that there might be some perinatal damages or functional disorders of the cerebral cortex.

Table 2 indicates that the development of agonistic muscle tension was impaired in the high fluoride group. Agonistic muscle tension is an indicator of the development of locomotive functions. Abnormality can occur if the brain is damaged or the development of the central nerve system is premature. Neurobehavioral capacity and agonistic muscle tension are closely related to long-term neuron-locomotive development. That is why there exist data that indicate decreased intelligence levels for grade school students in high fluoride areas.[5]

Fluoride ion has strong negative charge and can react with almost all cations. As the structure and the functions of the central nerve system are rather complicated, the mechanism of the toxicity of high fluoride to fetal nerve development remains unknown. It is reported that morphological changes have been observed in the brain tissues of fetuses in endemic fluoride regions. These tissues have higher number density and m/z ratio and lower average volume of nerve cells of the cerebral cortex, hippocampal pyramidal cells, cerebellar Purkinje cells, and undifferentiated nerve blast cells compared to fetuses from normal regions.

Observational results of ultra-microscopic structures indicate that, under electron microscopy, the mitochondria of nerve cells of the cerebral cortex, rough endoplasmic reticulum, and free ribosomes show decreases with obvious swelling of mitochondria, expansion of rough endoplasmic reticula of some nuclear isochromatin, aggregation along the edges, rupture of nuclear membranes, and spillage of the content of the cell nuclei. All these changes suggest slow development and poor differentiation of cerebral cortex neurons. Furthermore, a decrease in the number of synapses, destruction of membranes, and a decrease of mitochondria and vesicles and microtubules within mitochondria can all lead to decreased connections among neurons and abnormality of synapse functions.[6]

Y Yu et al. examined the ultrastructure of epithelia of thyroid follicles of fetuses in endemic fluoride regions and postulated that high fluoride may cause hypothyroidism in human fetuses, which, in turn affects the development of the central nervous system, resulting in retarded children in such regions.[7]

The toxic damage to the brain by high fluoride can also affect the transmitters and receptors of the cerebral nerves. It is reported that in the brains of fetuses from endemic fluoride regions, the levels of noradrenalin, 5-hydroxytryptamine and α1-receptor are lower than that of fetuses from non-endemic regions, while adrenalin level is significantly higher than that of fetuses from non-endemic regions. Such changes in transmitters and receptors are the material basis of the functional disorders of the nervous systems during fluoride poisoning.[8]

As a primary toxic material, fluoride can easily pass through the cellular membrane of various tissues and enter cells and thus inhibit DNA, RNA, the synthesis of proteins, and the activity of enzymes. High fluoride significantly lowers the total DNA and total RNA levels, which leads to lowered weight of the brain and delayed development of differentiation.[9]

The present observations indicate that fluoride, as a toxic material to nerve development, can have an adverse impact on the neurobehavioral development of neonates and can cause abnormal changes of neurobehavioral capability during the neonate period with a negative impact on the future development of both the body and intelligence of the neonate. Therefore, in endemic fluoride areas, great effort should be made to reduce fluoride level in the water. The quality of the water should be monitored after modification and the facility for the water modification should be managed accordingly. More attention should also be paid to improvement and protection of the environment so that the people in the endemic high fluoride regions can be protected from harm by high fluoride. Special attention should be paid to the health and protection of pregnant women. Expectant mothers should eat more foods high in calcium, vitamin D, and vitamin C to reduce the absorption of fluoride by the body and to promote the excretion of fluoride from the body to ensure better health for people in the high fluoride regions.[10]

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Sun Feng, director of the Women and Child Health Care Station, Zhaozhou County, Heilongjiang Province, and to Mr. Liu Yaowu of Zhaozhou County Epidemic Prevention Station for their support and coordination during the observation processes of this study. Our sincere thanks also go to Professor Ma Xiancai of the Second Clinical Hospital of Harbin Medical University for his help in the preparation and revision of this paper.

REFERENCES

1 Chen Bingheng, Qu Weidong. Health Assessment of Fluoride by WHO. *Chinese Journal of Endemiology* 2000;19:477-8.
2 Zang Zengyuan, Wang Jianguo, Zhou Minchun, et al. Study on the relationship between nutrition and bone deformation of fluoride bone patients. Chinese Journal of Endemiology, 2002;21:377-9.
3 Jin Hanzhen, Huang Demin, editors. Practical Pediatrics. 2nd ed. Beijing: People’s Health Press; 2000. p. 32.
4 Bao Xiulan, Gun Yizhen, Xue Guirong, et al. Neurobehavioral determination of normal neonates. Chinese Journal of Pediatrics 1986;24:341.
5 Wei Siqiong, Li Xiaoji. Investigation on the impact of long term intake of high fluoride on the growth and development of children. Sichuan Medicine 1990;11:46.
6 Du Li, Wang Changwu, Cao Xumao, et al. Impact of chronic fluoride toxicity on the brain development of human fetuses. Chinese Journal of Pathology 1992;21:218-20.
7 Yu Yanni, Dong Zhong, Liu Jialiu. Determination of visual parameters of thyroid glands of fetuses in fluoride endemic regions of Guizhou Province. Chinese Journal for the Prevention of Endemics 1998;13:148.
8 Yu Yanni, Yang Wenxiu, Dong Zhong, et al., Changes in transmitter and receptor of cerebral nerves in fetuses from fluoride endemic regions. Chinese Journal of Endemiology 1996;15:257-9.
9 Gong Zhizhong, Yu Yanni, Liu Jialiu, Investigation of cerebral DNA and RNA of rat fetuses from parents with chronic fluoride poisoning. Journal of Guiyang Medical College 1987;12: 104.
10 Wang Lihua, Sun Dianjun, Shi Yuxia, et al. Impact of protein and vitamin C on the changes in pathological morphology of various organs of fluoride poisoned rats through tea drinking. Chinese Journal of Endemiology 2002;21:254-7.

(Received on 09-16-2003; Editor: Zhang Zhaojun)