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The hazard of chromium exposure to neonates in Guiyu of China

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ABSTRACT

Guiyu is one of the most heavily chromium-polluted areas in China due to the presence of numerous electronic waste (e-waste) recycling sites in the region. In this study, we investigate the effect of umbilical cord blood chromium levels (UCBCLs) on neonates from Guiyu and discuss chromium-induced DNA damage of cord blood lymphocyte. Umbilical cord blood samples were collected from neonates of Guiyu (in 2006, n=100; in 2007, n=100) and the neighboring town of Chaonan (in 2006, n=52; in 2007, n=50) that is associated with the fishery. UCBCLs of the neonates were determined by graphite atomizer absorption spectrophotometer. Comet experiment was used to examine lymphocyte DNA damage. Questionnaires to gauge chromium exposure were administered to the mothers of the neonates. The mean UCBCLs of neonates in the Guiyu group in 2006 and 2007 were 303.38 μ g/L and 99.90 μ g/L with median 93.89 μ g/L and 70.60 μ g/L, respectively. We observed significant differences between the results in UCBCLs of neonates in Guiyu and the control group (P<0.01). There was no significant difference of UCBCLs in neonates between 2006 and 2007 in Guiyu (P>0.05). Higher levels of chromium in neonates were found to correlate with their mothers' exposure to e-waste recycling. There were significant differences in terms of DNA damage between the Guiyu group and the control group (P<0.05). There was a correlation between DNA damage and the UCBCLs of neonates (P<0.05). There is conclusive evidence that high UCBCLs in neonates exists in e-waste recycling areas in Guiyu and that e-waste recycling activity poses serious environmental problems. Chromium pollution is threatening the health of neonates around the recycling sites.

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1. Introduction

The production of electronic equipment is one of the fastest growing global manufacturing activities. Electronic equipment production and consumption has seen exponential growth in the last two decades. This development has resulted in an increase of e-waste. Due to their hazardous material contents, e-waste may cause environmental problems during the waste management

phase (Nnorom and Osibanjo, 2007). Since much e-waste is being exported to developing countries, many countries such as China, India, and Pakistan have had to deal with the issue of proper toxic waste disposal. Because of the lack of standardized rules in China relating to e-waste management, extensive emission of the pollutants from e-waste recycling occurs illicitly in some areas of Southern China. Millions of tonnes of these wastes are recycled in illegal workshops using environmentally unfriendly

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techniques including sorting, firing, incineration, and acidic wash (Huo et al., 2007; Liu et al., 2007).

Guiyu in Shantou, Guangdong Province, China is one of the popular final destinations of e-waste. Some preliminary investigations have found high levels of toxic heavy metals and organic contaminants in samples of dust, soil, river sediments, surface water and ground water in Guiyu resulting from the primitive e-waste recycling activities (Wang et al., 2005; Wang and Zhang, 2006; Zheng et al., 2008; Wong et al., 2007b). Of the many toxic heavy metals, chromium is one of the most widely used in electronic devices for various purposes including preventing rust and beautification of steel (Musson et al., 2006). Sampling chemical examination showed that chromium concentration in river sediments of Guiyu is 1338 times higher than the the soil-pollution-risk threshold issued by the U.S. environmental protection agency (Wong et al., 2007a).

Although chromium is an essential nutrient required for sugar and fat metabolism, excessive chromium can affect human oxidation-reduction and hydrolysis reactions. can lead to denaturation of protein, precipitation of nucleic acid, and interfere with normal enzymatic activity (Dingbang et al., 1995; Shi, 2003; Yugang et al., 2006). Generally, Chromium (Cr) exists in two stable states: Cr (III) and Cr (VI). The toxicity of chromium for the different valences is quite different. Cr (III) is a necessary trace element of the human body, but excessive intake of Cr (III) may bring about health damage. Cr (VI) is generally considered 1000 times more toxic than Cr (III) (Zhang and Jin, 2006). Cr (III) is not very soluble and is immobilized by precipitation as hydroxides. Cr (VI) is toxic, soluble, and easily transported in water resources (Kamaludeen et al., 2003). Chromium can cross the placenta, and may lead to excessive chromium exposure and influence the development of the fetus if blood chromium levels of mothers are raised by environmental pollution (Dingbang et al., 1995). In addition, chromium can lead to DNA damage, which may result in cancer-causing gene mutations. Many transition metals can lead to gene changes in the cell; chromium is one of them (Xiang, 1999).

The objective of this study is to investigate UCBCLs of neonates in the e-waste recycling town of Guiyu, to explore how chromium pollution may cause DNA damage of lymphocyte in peripheral blood of neonates, and to examine the influence of chromium pollution on the health of neonates. The study arose from the fact that unregulated e-wastes disposal has influenced the health of residents in Guiyu. The government and society should be very concern about this problem.

2. Materials and methods

2.1. Study of population

A total of 100 full-term neonates from the Department of Gynecology and Obstetrics of the local hospital of Guiyu were studied from July to October 2006. Concurrently, a control group of 52 full-term neonates from the Department of Gynecology and Obstetrics of Shantou Chaonan Minsheng Hospital (a neighboring town 20 km from Guiyu) was studied. And additional 150 umbilical cord blood samples were taken from neonates who were born in 2007, 100 from Guiyu and the 50 from control group. After informed consents were obtained from their parents or guardians, umbilical cord blood samples were

collected in all of neonates and questionnaires were administered to mothers. The study was approved by the Human Ethics Committee of Shantou University Medical College.

2.2. Collection and assay of samples

Cord blood of neonates was collected for the study of chromium levels in circulation of neonates. After cutting the umbilical cord of the neonates, 5 ml of umbilical cord blood was collected from the placenta using metal-free EDTAcontaining sealed glass automatic hemostix, and preserved in a refrigerator at -20 °C. Chromium levels in umbilical cord blood were determined in Central Laboratory of Shantou University Medical College by graphite furnace atomic absorption spectrometry (GFAAS), which consisted of a Shimadzu AA-660 AAS, a GFA-4B graphite furnace atomizer and an ASC-60G autosampler (Shimadzu Corporation, Kyoto, Japan) with the injection volume set at 15 µl. The main parameters used for chromium determination were a wavelength of 357.9 nm, current of 6 mA, a slit width of 0.5 nm, drying at 100 °C, ashing at 1200 °C, and atomization at 2500 °C. The accuracy of the method was controlled by recoveries between 95% and 107% from the spiked blood samples. If a blood sample showed increased chromium parents were recommended to contact their pediatrician.

2.3. SCGE assessing chromium damage to the DNA of cord blood lymphocyte

The SCGE (Single-cell gel electrophoresis, also called comet assay) is a new, rapid, simple and easily performed biochemical technique for assessing damage to the DNA of eukaryotic cells. The basic principle of the comet assay is the migration of DNA fragments on an agarose matrix under electrophoresis. When observed under the microscope, the cells show a cometlike appearance with a head (nuclear region) and a tail containing DNA fragments that have migrated towards the anode. A volume of 10 mL of cord blood cells was added to 120 mL of 0.5% low-melting-point agarose at 37 °C and deposited on histological slides previously covered with 1.5% normal-meting-point agarose in duplicate. They were immersed in 1% lysing solution (2.5M NaCl, 100 mM EDTA, 10 mM buffer Tris-HCl, pH 10, 1% sodium sarcosinate with 1% Triton X-100, 10% DMSO) for 1 h. After electrophoresis (under alkaline buffer solution, pH>13), they were fixed in absolute alcohol and stored until the moment of analysis. Samples were used for positive control and processed in vitro by hydrogen peroxide at 100 mM for 5 min in ice (in triplicate) in order to ensure assay reproducibility and sensitivity. The slides were stained by 100 μ l of propidium iodide (PI) (2 μ g/ml) and analyzed on a fluorescence microscope (Olympus) connected to a camera under a magnification of 400×. A total number of 100 cells (comets) per slide were analyzed. Two parameters were taken into account in order to estimate DNA damage: injury rate (tailing rate) and the lengths of tail.

2.4. Puerpera questionnaire

A questionnaire related to the exposure of chromium by pregnant women was used for a survey of mothers of neonates. The

	Group	N	Mother's age (years) (x̄±s)	body length (m)	Average	Apgar scores	Gender		Average	Delivery	
					body mass (kg) (x±s)		Male	Female	gestation ages (weeks) $(\bar{x}\pm s)$	Normal labor	Cesarean delivery
2006	Guiyu	100	27.21±5.24	0.50 ± 0.02	3.12±0.40	9.77±0.45	62%	38%	39.41±1.50	72%	28%
	Control	52	28.35 ± 4.95	0.50 ± 0.01	3.29 ± 0.52	9.79 ± 0.50	48.08%	51.92%	38.98 ± 1.54	57.69%	42.31%
	P		0.198	0.92	0.05	0.82	0.10		0.10	0.10	
2007	Guiyu	100	26.55 ± 5.15	0.50 ± 0.02	3.52 ± 3.56	9.72 ± 0.75	60	40	39.52 ± 1.69	60	40
	Control	50	25.86±3.98	0.50 ± 0.01	3.19 ± 0.44	9.72 ± 0.75	29	21	39.48 ± 1.39	32	18
	P		0.408	0.279	0.508	0.875	0.814		0.885	0.635	

P-values were calculated using Chi-square test for categorical data, and t test for quantitative data

questionnaire was divided into three parts. The first part was the general situation, which described the living and the working environment of neonates' parents. The main purpose was to study the relationship between environment chromium exposure and neonate chromium load. The second part described the health situation of pregnant women, and explored the others factors affecting neonates' chromium load. The third part described the general conditions of the neonates.

2.5. Statistical analysis

Data on several potential confounders (gender, mother's age, gestational time, and average body mass) were obtained from the questionnaires, and analyzed by using Chi-Square test and independent sample t tests. We used nonparametric M–U tests to compare chromium levels. Independent sample t-tests was used for comparisons of DNA damage of lymphocyte between Guiyu and the control group. Spearman correlation analysis was conducted to evaluate the relationship between UCBCLs in neonates and the DNA damage of lymphocytes. UCBCLs were regarded as dependent variables, and the 21 surveyed factors as independent variables for the Spearman correlation analysis. All statistical analyses were performed using SPSS13.0 software (SPSS Inc., Chicago, Illinois, USA). All significance tests were two-sided using P less than 0.05 as the level of statistical significance.

3. Results

3.1. General characteristics of neonates

A total of 302 cases of neonates were carried out for the study. There were 200 cases from Guiyu and 102 cases from the

Table 2 – The chromium levels of umbilical cord blood (μg/L) $\overline{X} \pm s$ Year Group Ν median Range 93.871) 3) 2006 100 Guiyu 0.45-6029.92 306.20 ± 845.78 Control 52 1 66-56 54 18 10 19 95 + 8 37 2007 Guiyu 100 20.16-630.00 99.90 ± 96.77 70.60^{2} Control 8.62-197.18 32.48 ± 32.74

Notes: compare with control group in the same year: 1) Z=-8.44, P<0.01; 2) Z=-8.08, P<0.01; compare between the year of 2006 and 2007 in Guiyu group: 3) Z=-0.999, P>0.05.

neighboring town. Table 1 summarizes the general characteristics of the neonates studied. No significant differences were found with respect to gender, mother age, gestational time, body length, body mass, Apgar scores, and delivery pattern between neonates in each group (P all greater than 0.05). Therefore these variables were not included in the final analyses. All of the neonates and mothers examined had no prenatal ischemic–anoxic history, and no history of diseases influencing development of the fetal central nervous system.

3.2. Chromium levels in umbilical cord blood of neonates

The mean of UCBCLs of neonates in Guiyu group in 2006 was 306.20 μ g/L, with a median 93.89 μ g/L. Nonparametric M–U tests were used to compare UCBCLs of neonates. Significant differences in UCBCLs of neonates could be found between Guiyu and the control group (P<0.01). The mean of UCBCLs of neonates in the Guiyu group in 2007 was 99.90 μ g/L, with a median of 70.60 μ g/L. Significant differences in UCBCLs of

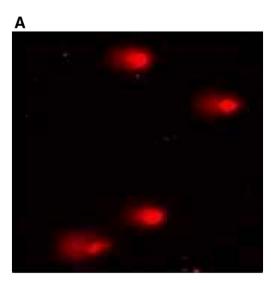
Table 3 – The Spearman correlations between UCBCLs in neonates and surveyed factors

Independent variable		Dependent variable			
	UCBCLs in 2006		UCBCLs in 2007		
	r _s	Р	r _s	Р	
Mother engaged in e-waste works	0.20*	0.01	0.35**	0.00	
The type mother engaged in works			0.31**	0.00	
Mother live in Guiyu	0.68**	0.00	0.51**	0.00	
The time mother live in Guiyu	0.58**	0.00	0.49**	0.00	
Residence in Guiyu during gestation period	0.68**	0.00	0.51**	0.00	
Be in Guiyu habitancy time during gestation period	0.65**	0.00	0.44**	0.00	
Father's occupation is with e-waste recycling	0.27**	0.00	0.42**	0.00	
The type fathers' engaged in works	0.44**	0.00	0.36**	0.00	
Mother living in the workshop of e-waste recycling			0.19*	0.02	
Mother was smoking			0.22*	0.01	
The time mother roaming in exposed site during gestation period everyday			0.23**	0.00	
Note:*P<0.05, **P<0.01.					

Table 4 - The correlations between body height/weight and cord blood chromium								
Year	Year Index		UCBCLs in neonates of Guiyu group		UCBCLs in neonates of Control group			
			rs	P	rs	P		
2006	Body length (cm)	50.46 ± 1.61	0.20	0.05	0.02	0.89		
	Body weight (kg)	3.18 ± 0.45	0.06	0.56	0.07	0.62		
2007	Body length (cm)	49.65 ± 1.54	0.11	0.26	-0.01	0.94		
	Body weight (kg)	3.41 ± 2.92	0.06	0.59	-0.10	0.48		

Table 5 - Effects of chromium on DNA damage in cord blood cells of neonates								
	N	The number of cell was analyzed	The cell contained by the tail	Injury rate (%)	Lengths of tail $(\overline{X} \pm s)$ (μ m)			
Guiyu group	35	3500	1162	33.20 ¹⁾	4.49±1.92 ²⁾			
Control group	20	2000	214	10.70	2.09±0.65			
Compare with control: 1) χ^2 = 343.47 P<0.01; 2) τ = 6.78 P<0.01.								

neonates also could be found between the two groups (P<0.01). Moreover, mean UCBCLs of neonates in 2007 was obviously lower than that in 2006. But there was not a significant difference of UCBCLs in neonates between the year of 2006 and 2007 in Guiyu group (P>0.05, Table 2).



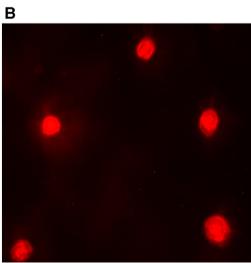


Fig. 1-Images of DNA damage of cord blood cells in neonates detected by comet assay (A: Guiyu group; B: Control group).

3.3. Correlations analysis between UCBCLs of neonates and surveyed factors

Taking UCBCLs in neonates as the dependent variable and surveyed factors as independent variables, as shown in Table 3, the Spearman correlate analysis indicated there was a correlation between UCBCLs in neonates in the 2006 group and 7 surveyed factors. There was also a correlation between UCBCLs in neonates in the 2007 group and 11 surveyed factors.

3.4. Correlations between chromium levels and body height and weight of neonates

Pearson correlation analysis showed there was no significant difference between UCBCLs in neonates and body height and body weight (P>0.05, Table 4).

3.5. The result of SCGE

Because of hematolysis, in total we gathered 55 samples of cord blood in neonates who were born in 2006 for Guiyu (n=35) and the control group (n=20) to do SCGE (Fig. 1). A total number of 100 cells per slide were analyzed; the percentages of the cells that have comet tail were calculated; the lengths of comet tail of each positive cell were measured with ocular micrometer [That is migration lengths of DNA fragments, which is from head (nuclear region) to terminal of tail]. Significant difference in injury rate and the lengths of tail could be found between Guiyu group and the control group (Table 5, P<0.05).

Table 6 – The correlations between cord blood chrome in neonates and DNA damage								
Independent	Dependent variable							
variable	The cell populations of comet		The cell lengths of tail					
	r _s	Р	rs	Р				
UCBCLs in neonates	0.95**	0.01	0.89**	0.00				
Note:**P<0.01.								

3.6. Correlations analysis between UCBCLs of neonates and DNA damage parameter

Spearman correlation analysis showed there was significant difference between UCBCLs in neonates and injury rate and the lengths of tail (P<0.05, Table 6).

4. Discussions

The chromium normal value varies among people. Generally, the total quantity of trivalent chromium in the adult body is 5 mg–100 mg. Chromium is widely distributed in the tissues, organs and body fluid in vivo. The chromium normal value in blood is 0 μ g/L–0.2 μ g/L. The chromium concentration of the tissue is 10–100 times that of blood chromium concentration (Anderson and Kozlovsky, 1985). In this study, the UCBCLs in neonates of Guiyu were many times higher than normal value. A significant difference in UCBCLs in neonates could be found between Guiyu group and the control group. Although the UCBCLs in neonates in 2007 had somewhat decreased compared with in 2006, there was no significant difference in UCBCLs in neonates could be found between 2006 and 2007 (P>0.05). This indicated that UCBCLs in neonates of Guiyu in 2007 were not noticeably improved.

Chromium in the human body originates from the diet, drinking water and air. The Spearman correlation analysis indicated the elevation of UCBCLs in neonates is associated with the mothers and fathers who had had exposure to chromium via different routes, smoking of pregnant woman and the time pregnant woman spent roaming in exposed sites. In Guiyu, nearly 80% of families in the town have engaged in e-waste recycling operations conducted by small scale family-run workshops. The processes and techniques used during the recycling activities in Guiyu were very primitive without pollution control measures (Huo et al., 2007). In house workshops, yards and open countryside, workers toil without protective goggles, masks or gloves dismembered the detritus of modernization. Armed mostly with small, simple tools, workers take apart these old apparatuses, strip wires and cable for the copper they contain, melt the lead solder from circuit boards over coal stoves, place chips in open acid baths to separate precious metals, including the tiny quantities of gold and palladium they contain. The waste acid is then dumped into nearby streams (Brigden et al., 2005); grade plastics quality by their nose, burn scrap wires and transformers to separate metal from rubber coating. Hence, during the course of recycling, significant quantities of lead, mercury, cadmium, PVCs, flame retardants, chromium VI and other hazardous substances contained in electronic sets become environmental risks. Several studies have reported the soaring levels of toxic heavy metals and organic contaminants in samples of dust, soil, river sediment, surface water, and groundwater of Guiyu (Brigden et al., 2005; Puckett et al., 2002; Wang et al., 2005; Wang and Zhang, 2006; Yu et al., 2006). Sampling chemical examination showed that concentration of chromium in Guiyu air, soil and sediments were higher than that of other cities (Wong et al., 2007a). The influence of smoking on health not only is because of the Nicotine, but it also causes harmful trace elements, including chromium, to increase in the human body. Chromium concentration in the tissue fluid of smokers is higher than nonsmokers. There is a close correlation between chromium concentration and lung cancer and emphysema. We can see e-waste everywhere in Guiyu Street. Vehicles moving on the streets can cause deposition dust to mix with the harmful metals including chromium and scatter in the air. So the chromium concentration in the air dust in the streets of regions like Guiyu should be higher than other areas. There was a correlation between UCBCLs in neonates and the time pregnant women spent roaming in exposed sites.

Hexavalent chromium is a well-known highly toxic metal, considered a priority pollutant (Mohan and Pittman, 2006). Both toxicokinetic and genotoxicity studies indicate that a portion of an orally administered dose of hexavalent chromium is absorbed and gets into the cells of several tissues, causing DNA damage (Standeven and Wetterhahn, 1989). Cr (VI) can generate Cr-DNA binary (mono) adducts, Cr-DNA ternary adducts, DNA protein cross links (DPCs), bi-functional (DNA interstrand cross links (ICLs)) adducts, single-strand breaks (SSBs) and oxidized bases etc. Cr (VI) exposure elicits a classical DNA damage response within cells including activation of the p53 signaling pathway and cell cycle arrest or apoptosis. Moreover, Cr (VI) also induces the ATM-dependent DNA damage response pathway which is paradoxically required for both apoptosis and survival after Cr (VI) insult (O'Brien et al., 2003). In Guiyu, discharge of Cr waste from many e-wastes such as leather tanning, electroplating and metallurgy has led to large-scale contamination of land and water. Although Cr (VI) undergoes rapid reduction to Cr (III) after traversing the cell membrane by non-specific anionic transporters, a lot of Cr (VI) were absorbed and resulted in DNA damage, which become potential risk of many diseases. Our study has demonstrated that the chromium exposure may impair DNA of cord blood lymphocyte of neonates in Guiyu as evidenced by the correlation between the DNA damage of lymphocyte and UCBCLs of neonates. The significant positive correlation between the DNA damage of lymphocyte and UCBCLs of neonates suggests that the impairment of DNA is at least in part due to the high levels of chromium contents in neonates. Of course, there were many other poisonous heavy metals and organics influencing DNA damage of lymphocyte in neonates, but our study design has not examined if DNA damage was due to the increased level of other elements. This study showed that chromium pollution may influence the neonates' health in Guiyu, which may be severe result in the

In conclusion, high UCBCLs of neonates in Guiyu are found in this study. The chromium levels may be due to environmental contamination, which is caused by the primitive e-waste recycling activities in the local region. Although the UCBCLs of neonates in the year of 2007 had somewhat decreased compared with that of 2006, it was still a serious threat to neonates' health around the e-waste recycling area. Based on our study, we also have concluded that neonates in Guiyu who exposed to higher chromium levels due to the recycling of e-waste have impaired DNA of cord blood lymphocyte. It is necessary to pay more concern on increasing public awareness about the adverse effects of primitive e-waste recycling activities. Policies that help to mitigate the environmental chromium levels should be made.

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