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| Keywords: | hexavalent chromium, oxidative stress, oxidative damage, DNA damage, duration of residence |
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# Oxidative Stress and DNA Damage in a Long-Term Hexavalent Chromium-Exposed Population in North China: A Cross-sectional Study

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**Objectives**

The International Agency for Research on Cancer (IARC) classifies Hexavalent chromium [Cr(VI)] as a carcinogen. Cancer mortality is reported higher in the hexavalent chromium contaminated regions. Scientists have recommended studying the health impact of living in contaminated regions. This study aims to evaluate the health risk the population living in hexavalent chromium contaminated areas confronted with.

**Design**

We conducted a cross-sectional study in rural areas in northern China. Malondialdehyde (MDA), Glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were used as oxidative stress parameters and 8-hydroxy-2 deoxy guanosine (8-OHdG) as DNA damage parameter. We collected each subject's information about the socio-demographic characteristics, the lifestyle and the duration of residence by a questionnaire in local villages. The biological samples were collected the same day as the survey. We used t test and chi-square test for univariate analysis and multiple general linear regression analysis for multivariate analysis.

**Participants**

The sample included 319 exposed and 307 unexposed people. 447 females and 179 males were enrolled for this study. These participants met the following criteria: a) living in this district more than 10 years; b) adults older than 18 years; c) no occupational chromium exposure.

**Results**

Our study revealed that the MDA level ($p<0.001$), CAT level ($p<0.001$), GSH-Px level ($p<0.001$) and urinary 8-OHdG level ($p=0.008$) in the exposed group were significantly higher than the same four parameters in the unexposed group. However, SOD level was significantly lower in the exposed group compared with the SOD level in the unexposed group ($p<0.001$). There was an interaction effect between serum GSH-Px activity and smoking, and between serum GSH-Px activity and alcohol ($p<0.05$). Longer residence in the exposed area could increase the oxidative levels ($p<0.05$).

**Conclusions**

The findings of this study show elevated oxidative stress and DNA damage among persons exposed to hexavalent chromium.

**Key word**: hexavalent chromium, oxidative stress, oxidative damage, DNA damage, duration of

residence.

**Strengths and limitations of this study:** 1.To the authors' best knowledge, this is the first paper to study the relationship between the hexavalent chromium exposure and oxidative levels in non-occupational hexavalent chromium exposed populations.

2. The main limitation of our study is that individual-exposed data were not obtained.

3. In addition, the demographic characteristics' homogeneity of the exposed and unexposed groups is not satisfactory on account of the difference between the whole populations of the two area.

## 1 Introduction

Hexavalent chromium compounds are commonly found in industrial settings such as chromite ore mining, pigment production, leather tanning, manufacture of wood preservatives, and from anticorrosive agents in cooking products.[1] Heavy metals from anthropogenic sources can be transported in the air, deposited on the soil surface and then penetrate into the water.[2 3] High concentrations of heavy metals in soil may correlate with a high concentration in plants.[4] People living near the contaminated areas may be confronted with health risks due to the heavy metal concentrations in food or water.[5] The general population may be exposed to chromium through contaminated water, food or air.[6]

Hexavalent chromium is considered as a carcinogen according to the IARC monographs on the evaluation of carcinogenic risks to humans.[7-10] Based on numerous occupational epidemiology studies, the inhalation of hexavalent chromium [Cr(VI)] correlates with increased lung cancer risk.[9] Numerous epidemiological studies report an increased risk of cancer morbidity, especially for gastrointestinal cancer, in populations exposed to hexavalent chromium.[7 11] For this reason, hexavalent chromium contamination may pose a serious threat to population health.

The toxicity of hexavalent chromium and carcinogenicity is possibly related to increased oxidative stress.[12] When Cr(VI) is reduced to a lower oxidative state, many reactive oxygen species(ROS) are formed. Therefore, one of the most important negative effects caused by extraneous Cr (VI) is the formation of ROS during the reduction of Cr (VI) in cells.[13] The generated hydroxyl radicals are able to react with DNA bases. For this reason, the best described substance is 8-hydroxyguanosine (8-OH-dG), a good marker for oxidative damage in an organism.[14] The reduction of the extra ROS can be accomplished through enzymatic reactions and non-enzymatic

reactions. Oxidative stress results from an imbalance between the production of free radicals and the antioxidant defense system, leading to a reduced capacity to detoxify free radicals and repair damage.[15] The attack of free radicals on cellular components has been studied in various pathological conditions such as in cardiovascular disease and cancer.[16-18]

Due to the industrial expansion of the mid-20$^{th}$ century, the western suburban regions of Jinzhou city have been polluted by hexavalent chromium.[8] Multiple studies have shown that occupational exposure to hexavalent chromium induces changes in the levels of oxidative stress and oxidative damage. However, there is only a limited amount of human data on the environmental exposure of hexavalent chromium in terms of oxidative stress and oxidative damage. People living close to an alloy plant could be exposed to Cr(VI) not only by the respiratory route, but also through the digestive route and skin contact. The mortality rates of stomach cancer and lung cancer in regions where water was contaminated by hexavalent chromium [Cr(VI)] are much higher in comparison with the regions with no water contamination.[7 11] All the Cr(VI)-polluted areas in this study are along the Nver river, which have been polluted by alloy plants. Previous studies have shown that the highest chromium concentration in wells in this area were 20mg/L.[11] A ferrochromium factory was established in 1960 and since then the population living near the factory has been exposed to hexavalent chromium. After a long time exposure, a series of health risks may be induced in these populations. Therefore, this research mainly aims to study whether exposure to hexavalent chromium could induce changes in oxidative stress and oxidative damage levels.

## 2Materials and Methods

### 2.1 study design and population

We conducted a cross-sectional study in the villages of Jinzhou city located in the Liaoning province, to evaluate the levels of oxidative stress and oxidative damage caused by hexavalent chromium. 447 females and 179 males were enrolled for this study. These participants met the following criteria: a) living in this district more than 10 years; b) adults older than 18 years; c) no occupational chromium exposure. Subjects were divided into exposed and unexposed groups based on geographical position, historical data and environmental chromium level. The exposed district was comprised of Jinchangbao village, Nverhe village and Qiantanghe village, and the unexposed district was comprised of Baolin village, Yijiayu village, and Shifobao village. All the

exposed villages were along the contaminated river, less than 10 kilometers away from the plant, with a high chromium levels in environment. The unexposed villages were at least 50 kilometers away from the plant, with a relatively low chromium levels in environment (Figure 1, Table 1). All individuals enrolled in this study signed the informed consent form for this study. Ethical clearance for the conduct of the study was obtained from the ethical committee, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences.

**2.2 Questionnaire survey**

Specially trained postgraduate and graduate students were in charge of the participants' structured questionnaire presented face-to-face. The questionnaire was designed to collect information about socio-demographics (sex, birth date, survey date, education level, occupation status, personal income, marital status and duration of residence), lifestyle (e.g. smoking status, alcohol drinking) and whether the participant had occupational exposure to hexavalent chromium and other related issues.

**2.3 Blood and urine sample collection**

Blood samples were collected after overnight fasting. Serum samples were collected into non-anticoagulation tubes and obtained by centrifugation at 3500 rpm for 10 minutes to precipitate the cellular components from the blood specimen. 10mL early morning urine samples were collected into bacteria-free CentrifugeTubes. The sample was transported in an ice box to guarantee the quality of the samples. Subsequently, all the experiments were conducted within 24 hours, and the remaining samples were stored at −80°C for later analysis.

**2.4 MDA concentration in serum, serum CAT, SOD, GSH-Px activity measurements**

MDA concentration in serum, serum CAT activity, serum SOD activity and serum GSH-Px activity were respectively determined by Malondialdehyde (MDA) assay kit (TBA method), Catalase (CAT) assay kit (Visible light), Total Superoxide Dismutase (T-SOD) assay kit (Hydroxylamine method), and Glutathione Peroxidase (GSH-Px) assay kit (Colorimetric method). All above kits were supplied by Nanjing Jiancheng Bioengineering Institute (China). The absorbance of MDA and SOD for each sample were measured with a Tecan sunrise Microplate Reader (Tecan, Switzerland), and the absorbance of CAT and GSH-Px for each sample were measured with 723 spectrophotometer (V-5100, Metash, Shanghai).

**2.5 Urinary 8-OHdG assay**

The concentration of 8-OHdG was chosen to determine the content of urinary 8-OHdG using

an ELISA kit (JaAICA, Japan, 8-OHdG EIA kit) and measured with a Tecan sunrise Microplate Reader (Tecan, Switzerland). In order to minimize the influence of urinary density difference among subjects, the 8-OHdG level was regulated with urinary creatinine (Cre). The concentration of urinary Cre was determined by ELISA assay with a commercial kit from Roche Pharmaceutical Ltd. (Switzerland) and measured with a Roche P800 automatic analyzer (Roche Pharmaceutical Ltd, Switzerland).

**2.6 The collection and testing of Environmental samples**

The environmental samples were all collected in the studied area at the same time when conducting the survey. We collected groundwater samples in 7 or 8 meters-deep wells in the yard of the participants' house and soil samples in the fields' surface soil. The air samples were collected 5 days, 24 hours a day in three exposed villages and three unexposed villages with changing the sample film every 24 hours. All samples were stored in a refrigerator at 4 °C for further analysis in the laboratory. The concentration of hexavalent chromium in groundwater was tested by Diphenyl carbazide spectrophotometric method with the detection limit of 0.004mg/L.[19] If the detection result was under the limit, we used half of the detection limit as outcome for the statistic analysis. The total chromium level in soil and air were tested by the method of atomic absorption spectrophotometry and Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).[20 21] **2.7 Quality control**

All the investigators would explain the exact meaning of each question to the subjects given that most of the subjects didn't have a high education level. During the investigation, investigators would check the integrity of the questionnaire and make sure that the blood and urine samples were collected. To ensure that every individual had a unique identification to match their questionnaire and biological samples, a standard coding rule was used. The laboratory staff followed the protocol of the kit strictly to conduct analysis. The absorbance of a sample was measured three times, using the average as the final value. If the fluctuation was more than 50 percent among the three absorbance, the sample analysis was re-conducted. The testing of the environmental samples were strictly followed the protocol of the method.

**2.8 Statistical analysis**

Epidata3.1 was used to input the original data collected from questionnaires with the rule of double-entry and logistical error check in order to ensure accuracy. Categorical variables were

presented as numbers with proportions and continuous variables as median with IQR or mean with standard deviations. The unpaired t test was used to compare two mean values and chi-square test was used to compare categorical variables. If the data did not obey normal distribution, Wilcoxon rank sum test was used. Subsequently, multiple general linear regression analysis was performed to analyze the main factors that affected the level of oxidative stress and oxidative damage. To deal with skewed data, we used the log-transformation on the variable 8-OHdG in our analysis. The multivariate model did not use occupation and marital status because these two variables had less than 10% of cases in a group. All the expected and observed cases of variables used in the multivariate model was more than 5 in every bivariate cell of the 2-way tables. To further explore the relation between hexavalent chromium exposure and oxidative stress and oxidative damage, we conducted stratified analysis according to age, sex, smoking status, alcohol drinking and education level considering the heterogeneity between the two groups. We also did stratified analysis according to disease status on account that some diseases may have an effect on oxidative levels. Meanwhile, interaction terms of grouping variable and stratifying variables were also added into the models to explore potential interactions. Since age at first exposure and period of residence may have an effect on the oxidative levels, we conducted a stratified analysis in the subgroup of first exposure before and after 18 years old to explore the relationship between the duration of residence and oxidative levels in exposure group. Statistical significance was defined as $p < 0.05$ (two tail). All analyses were performed using SAS (version 9.4, Cary, NC, USA).

**3 Results**

Table1 presents the chromium level in groundwater, soil and air. Hexavalent chromium is not detected in all the groundwater samples of unexposed area, while the maximum concentration in the samples of exposed area have reached 2.5mg/L, and concentration of hexavalent chromium in the groundwater is significantly different between the two areas ($p=0.0017$). The total Cr concentration in soil and in air of exposed area are both significantly higher than unexposed area with a p value of <0.001 and 0.015.

**Table 1.Chroimum level in study area in 2016**

| Cr concentrations | Exposed area | | Unexposed area | | P* |
|---|---|---|---|---|---|
| | n | Median ( Min, Max ) | n | Median ( Min, Max ) | |
| Groundwater (mg/L)† | 13 | 0.002 ( 0.002 , 2.50 ) | 18 | 0.002 ( 0.002 , 0.002 ) | 0.0017 |
| Soil (mg/kg) | 45 | 69.53 ( 48.66 , 417.12 ) | 30 | 29.22 ( 20.06 , 41.11 ) | <0.001 |
| Air (ng/m$^3$) | 15 | 19.30 ( 10.14 , 82.88 ) | 15 | 13.12 ( 4.96 , 18.68 ) | 0.015 |

† Hexavalent chromium concentrations

* Wilcoxon rank test was used to compare exposure and non-exposure group.

Table 2 presents the demographic characteristics of the exposed and unexposed samples. A total of 626 participants including 319 exposed villagers and 307 unexposed villagers are recruited in this study. Table 2 shows there is no significant difference in occupation, marital status, and personal income between exposed and unexposed groups. However, significant group differences ($p<0.05$) are found with respect to age, sex, education level, smoking status and alcohol drinking. Specifically, subjects in the exposed group are older, more likely to be female, more likely to have higher education, and less likely to smoke or drink. The exposed group shows significantly higher concentration of serum MDA ($p=0.0003$), serum CAT activity ($p<0.0001$), serum GSH-Px activity ($p<0.0001$) and concentration of urinary 8-OHdG as compared to the unexposed group (Table 2) .However, the SOD activity of the exposed group is significantly lower compared to the unexposed group ($p<0.0001$) (Table2). Multiple regression analysis shows that serum MDA concentration ($p=0.0001$), serum CAT activity ($p<0.0001$), serum GSH-Px activity ($p<0.0001$) and concentration of urinary 8-OHdG ($p=0.0117$) are significantly higher in the exposed group compared to the unexposed group after adjusting for gender and age (Table3, Model 1). After further adjustment for smoking status, alcohol drinking, marital status, occupation, personal income, and education level, results remain significant for serum MDA concentration ($p=0.0001$), serum CAT activity ($p<0.0001$), serum GSH-Px activity($p<0.0001$) and concentration of urinary 8-OHdG ($p=0.0075$) (Table 3, Model 2). Multiple regression analysis shows that serum SOD activity is significantly ($p<0.0001$) lower in the exposed group than the unexposed group after adjusting for sex and age (Table3, Model 1), and remains significantly lower after further adjustment for smoking, alcohol, marital status, occupation, personal income, and education ($p<0.0001$) (Table3, Model2). In model 1, the serum concentration of urinary 8-OHdG between different ages is also significant in Model 1 and Model 2 with p values of <0.001

and 0.002 (data shown in the Appendix A and Appendix B).

**Table 2. Demographic characteristics and oxidative parameters of the study participants.**

| Variables | All | Hexavalent chromium | | *P* value[2] |
|---|---|---|---|---|
| | (n=626) | Exposure | None-exposure | |
| No. of subjects | 626 | 319 | 307 | |
| Age (y) [1] | 60.34 ± 10.57 | 61.21 ± 9.36 | 59.44 ± 11.64 | 0.0377 |
| Sex [n (%)] | | | | <0.0001 |
| Male | 179 ( 28.59 ) | 69 ( 21.63 ) | 110 ( 35.83 ) | |
| Female | 447 ( 71.41 ) | 250 ( 78.37 ) | 197 ( 64.17 ) | |
| Education [n (%)] | | | | <0.0001 |
| Primary school or lower | 342 ( 54.63 ) | 146 ( 45.77 ) | 196 ( 63.84 ) | |
| Middle school or higher | 283 ( 45.21 ) | 173 ( 54.23 ) | 110 ( 35.83 ) | |
| Occupation [n (%)] | | | | 0.0665 |
| Peasant | 589 ( 94.09 ) | 295 ( 92.48 ) | 294 ( 95.77 ) | |
| Others | 37 ( 6.91 ) | 24 ( 7.52 ) | 13 ( 4.23 ) | |
| Smoking status [n (%)] | | | | 0.0049 |
| No | 458 ( 73.16 ) | 249 ( 75.86 ) | 209 ( 68.08 ) | |
| Yes | 168 ( 26.84 ) | 70 ( 21.94 ) | 98 ( 31.27 ) | |
| Alcohol drinking [n (%)] | | | | 0.0033 |
| No | 511 ( 81.63 ) | 275 ( 86.21 ) | 236 ( 77.85 ) | |
| Yes | 114 ( 18.21 ) | 44 ( 13.79 ) | 70 ( 22.80 ) | |
| Marital status [n (%)] | | | | 0.5424 |
| Married | 580 ( 92.65 ) | 298 ( 93.42 ) | 282 ( 91.86 ) | |
| Others | 45 ( 7.19 ) | 21 ( 6.58 ) | 24 ( 7.82 ) | |
| Personal income (yuan)[n ( %)] | | | | 0.9049 |
| <2000 | 285 ( 45.53 ) | 148 ( 46.39 ) | 137 ( 44.63 ) | |
| 2000-5000 | 152 ( 24.28 ) | 76 ( 23.82 ) | 76 ( 24.76 ) | |
| >5000 | 189 ( 30.19 ) | 95 ( 29.78 ) | 94 ( 30.62 ) | |
| MDA (nmol/mL) [1] | 3.40 ± 0.99 | 3.55 ± 0.87 | 3.25 ± 1.08 | 0.0003 |
| SOD (U/mL)[1] | 61.72 ± 16.22 | 54.52 ± 15.42 | 69.06 ± 13.53 | <0.0001 |
| GSH-Px (U/mL)[1] | 174.31 ± 73.28 | 196.04 ± 79.89 | 152.79 ± 58.76 | <0.0001 |
| CAT (U/mL)[1] | 3.94 ± 1.58 | 4.64 ± 2.85 | 3.21 ± 1.83 | <0.0001 |
| 8-OHdG(μg/mmol-Cre) [3] | 1.16 | 1.36 | 0.92 | <0.0001 |
| | ( 0.69, 2.06 ) | ( 0.81, 2.21 ) | ( 0.58, 1.65 ) | |

[1] Mean ± SD (all such values) [2]A Student's t test was used for continuous variables, and a chi-square test was used for categorical variables. [3] Median ($P_{25}$-$P_{75}$), Wilcoxon rank test was used to compare exposure and non-exposure group.

**Table 3. Multiple factors regression analysis on oxidative parameters and chromium in exposure and non-exposure**

| Parameter | Model | exposure (Lsmean ± SE) | Non-exposure (Lsmean ± SE) | p |
|---|---|---|---|---|
| MDA ($nmol\text{-}mL^{-1}$) | model1* | 3.62 ± 0.06 | 3.29 ± 0.06 | 0.0001 |
| | model2※ | 3.65 ± 0.07 | 3.33 ± 0.06 | 0.0001 |
| SOD ($U\text{-}mL^{-1}$) | model1 | 53.87 ± 0.90 | 68.80 ± 0.85 | <0.0001 |
| | model2 | 54.06 ± 1.02 | 68.99 ± 0.95 | <0.0001 |
| GSH-Px ($U\text{-}mL^{-1}$) | model1 | 197.47 ± 4.44 | 153.77 ± 4.12 | <0.0001 |
| | model2 | 194.99 ± 5.00 | 149.33 ± 4.64 | <0.0001 |
| CAT ($U\text{-}mL^{-1}$) | model1 | 4.86 ± 0.15 | 3.31 ± 0.14 | <0.0001 |
| | model2 | 4.77 ± 0.17 | 3.17 ± 0.16 | <0.0001 |
| 8-OHdG) †(ng/μmol-Cre) | model1 | 0.11 ± 0.03 | 0.02 ± 0.02 | 0.0117 |
| | model2 | 0.12 ± 0.03 | 0.03 ± 0.03 | 0.0075 |

*model1 is adjusted for sex, age ※model2 is adjusted for sex, age, personal income, education, smoking and alcohol use. And p value of every model is less than 0.05. †logarithm-transformed to adjust the distribution

In a stratified analysis, the difference of serum MDA concentration between exposed and unexposed groups is significant in subgroups defined by age, sex, smoking status, alcohol drinking and education level (Table 4). Serum CAT activity shows significant differences between exposed and unexposed groups by age, sex, smoking status, alcohol drinking and education (Table4). The stratified analysis also shows that the difference of serum SOD activity between exposed and unexposed groups is significant in all of the subgroup analyses by age, sex, smoking status, alcohol drinking and education. The difference of serum GSH-Px activity between exposed and unexposed groups is significant by age, sex, smoking status, alcohol drinking and education. An interaction effect is observed between serum GSH-Px activity and smoking status, and between GSH-Px activity and alcohol drinking (Table4). As for the urine 8-OHdG, the stratified analysis indicates a significant difference between exposed and unexposed groups in age, sex, smoking status, and alcohol drinking (Table4). The mean and standard deviation of participants' duration of residence in exposure group is 45 years and 13years. Duration of residence have a positive association with the oxidative levels (Table 5). The serum CAT activity (p=0.0466) and the urine 8-OHdG concentration (p=0.0242) become higher along with the increase of resident years in the subgroup of first exposure age before 18. Besides, the serum GSH-Px activity (p=0.0369) is higher

along with the increase of resident years in the subgroup of first exposure after 18.

**Table 4.Oxidative parameters average level according to exposure status in subgroups (Lsmeans±SE(n))**

| | | MDA(nmol/ml) | | SOD(U/ml) | | CAT(U/mL) | | GSH-Px(U/mL) | | Lg(8-OHdG)(μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed |
| Age | <60 | 3.79±0.11** | 3.44±0.10 | 52.44±1.59** | 68.84±1.43 | 4.82±0.28** | 3.28±0.25 | 189.47±7.87** | 150.49±7.00 | 0.08±0.04* | -0.02±0.04 |
| | | (134) | (152) | (138) | (152) | (138) | (152) | (131) | (152) | (127) | (135) |
| | ≥60 | 3.53±0.09** | 3.25±0.09 | 54.68±1.29** | 68.42±1.36 | 4.55±0.21** | 2.93±0.21 | 200.54±6.61** | 145.41±6.56 | 0.19±0.04 | 0.10±0.04 |
| | | (172) | (151) | (173) | (151) | (174) | (151) | (169) | (149) | (154) | (126) |
| Sex | male | 3.68±0.12 | 3.50±0.10 | 53.99±1.63** | 67.65±1.30 | 5.54±0.33** | 3.46±0.26 | 196.89±8.92* | 161.25±7.11 | 0.13±0.04* | -0.05±0.04 |
| | | (68) | (109) | (68) | (109) | (68) | (109) | (67) | (108) | (63) | (96) |
| | female | 3.46±0.13** | 3.12±0.13 | 55.78±1.98** | 71.17±1.98 | 4.49±0.30** | 3.04±0.30 | 189.16±9.63** | 138.98±9.43 | 0.10±0.04 | 0.04±0.04 |
| | | (238) | (194) | (243) | (194) | (244) | (194) | (233) | (193) | (218) | (165) |
| Smoking | no | 3.74±0.10** | 3.35±0.10 | 53.48±1.52** | 68.95±1.49 | 4.94±0.25** | 3.24±0.25 | 205.03±7.29**† | 148.22±7.08 | 0.10±0.04* | -0.02±0.04 |
| | | (238) | (207) | (242) | (207) | (243) | (207) | (234) | (206) | (221) | (177) |
| | yes | 3.50±0.13 | 3.40±0.11 | 55.46±1.75** | 68.84±1.50 | 4.70±0.28** | 3.29±0.24 | 172.10±9.25 | 155.54±7.77 | 0.09±0.05 | 0.07±0.05 |
| | | (68) | (95) | (69) | (96) | (69) | (96) | (66) | (95) | (60) | (84) |
| Alcohol | no | 3.62±0.08** | 3.25±0.08 | 53.20±1.24** | 68.74±1.21 | 5.07±0.21** | 3.46±0.20 | 198.38±5.90**※ | 146.20±5.70 | 0.07±0.04* | -0.02±0.03 |
| | | (263) | (234) | (268) | (234) | (269) | (234) | (260) | (233) | (240) | (201) |
| | yes | 3.40±0.19 | 3.34±0.16 | 56.95±2.47** | 68.56±2.18 | 4.73±0.37** | 3.17±0.33 | 175.01±13.78 | 157.48±11.84 | 0.12±0.07 | 0.04±0.06 |
| | | (43) | (69) | (43) | (69) | (43) | (69) | (40) | (68) | (41) | (60) |
| Education | Primary or lower | 3.53±0.11 | 3.31±0.09 | 53.07±1.51** | 69.16±1.26 | 4.89±0.24** | 3.40±0.20 | 199.52±6.98** | 151.10±5.82 | 0.14±0.05 | 0.05±0.05 |
| | | (139) | (194) | (142) | (194) | (142) | (194) | (141) | (193) | (126) | (165) |
| | Middle or higher | 3.76±0.09** | 3.28±0.10 | 54.82±1.43** | 68.32±1.59 | 4.59±0.24** | 2.87±0.27 | 192.31±7.51** | 150.22±8.16 | 0.10±0.03 | 0.02±0.04 |
| | | (167) | (109) | (169) | (109) | (170) | (109) | (159) | (108) | (155) | (96) |

*p<0.05 versus unexposed population **p<0.001 versus unexposed population †p=0.0357 of interaction effect between smoking and exposure status ※p=0.006 of interaction effect between alcohol and exposure status

**Table 5.Analysis duration of residence (years) and oxidative parameters by different age at first exposure in the exposure group (Lsmeans±SE)**

| Age at first exposure(years) | GSH-Px※ | | | | CAT※ | | | | 8-OHdG†※ | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≤38 | 38-55 | >55 | p* | ≤38 | 38-55 | >55 | p* | ≤38 | 38-55 | >55 | p* |
| ≤18 | 180.14 ± 38.01 | 167.46 ± 15.12 | 180.94 ± 11.32 | 0.6902 | 3.07 ± 1.04 | 4.23 ± 0.44 | 4.94 ± 0.32 | 0.0466 | -0.22 ± 0.15 | 0.10 ± 0.06 | 0.16 ± 0.05 | 0.0242 |
| >18 | 196.73 ± 10.61 | 202.27 ± 16.42 | 257.21 ± 10.14 | 0.0369 | 5.00 ± 0.48 | 5.10 ± 0.73 | 5.67 ± 0.89 | 0.5725 | 0.10 ± 0.05 | 0.12 ± 0.08 | 0.17 ± 0.05 | 0.5417 |

*p for trend †logarithm-transformed to adjust the distribution ※The model is adjusted for age, sex, personal income, education, smoking status and alcohol use

## 4 Discussion

When the environmental Cr concentration (underground water, soil and air) is fairly high in exposed region, people living in the regions are generally at high risk to be exposed to hexavalent chromium. Since 1970s, the villagers in the exposed area had stopped using the groundwater as drinking water thanks to the government's water improvement project, while some of the villagers still use the groundwater to irrigate the fields and do some washing. Moreover, they would contact the high chromium concentration soil with their hands and skin when cultivating. Thus, the villagers in exposed area may contact hexavalent chromium through skin or hand-to-mouth route. [5] Besides, the chromium in the air provides a respiration way for the exposed villagers to contact higher concentration chromium, which is considered posing a threat to people's health. [8] Therefore, through all above exposure pathways, people living in the chromium-exposed area may have a health risk compared to those living in unexposed area.

In this study, our results indicates that after adjusting the possible confounders, exposure to hexavalent chromium can pose damage to lipids and DNA for people living nearby the pollutant sources. In addition, exposure to hexavalent chromium affects the antioxidant system, such as the activation of the antioxidant system or damaging the antioxidant system.[17 22] Besides the significant differences between the exposed and unexposed group, additional factors affects the results, such as sex, age, smoking and alcohol use. Moreover, longer residence in exposed areas may increase the health risk.

Lipid peroxidation has been suggested to play a key role in many biological processes, and MDA has long been used as a marker for secondary products of lipid peroxidation.[23] Many studies have shown that there is a significant increase of MDA in chromium exposed groups compared to unexposed groups both in humans and animals, which may indicate that chromium exposure results in the depletion of the antioxidant defense elements, subsequently causing lipid peroxidation.[24-27] In our study, we find that the MDA concentration of the exposed group is significantly higher than that in the unexposed group after adjusting for sex and age or even in the full model. The evaluated MDA concentration indicates increased rate of oxidative stress levels in the lipid in exposed populations. However, the results of the stratified analysis show that the MDA concentration is not significantly affected by exposure to hexavalent chromium in the subgroup that smokes or consumes alcohol. On the other hand, exposure to hexavalent chromium still affects the non-smoking population and those that do not consume alcohol. Many studies have shown that after adjusting for potential confounders, smoking and drinking can elevate the concentration of MDA in both animal models and in humans.[28 29] However, we do not find significant association either between smoking and MDA concentration or between alcohol and MDA concentration.

In our view, this lack of correlation could be mainly due to the strong influence of hexavalent chromium, which covers the effect that smoking and alcohol have on the MDA concentration. There have been some discrepancies in the concentration of MDA in gender in previous studies. In trivalent chromium exposed populations, the MDA concentration is higher in females than in males.[25] However, in normal populations the concentration of MDA is lower in females then in males.[30] In this study, the MDA concentration in females is lower than that in males, which is consisted in both the exposed and unexposed groups. This result is consistent with results in the studies done on normal populations.

Reactive oxygen species are formed when Cr(VI) reduced to a lower oxidation state, and the free radicals may attack DNA thereby disrupting cellular functions and integrity.[27] Thus DNA damage produces alterations in DNA, strand breaks and DNA-protein crosslinks. 8-OHdG is a major oxidative adducts formed by radicals inducing damage to DNA. [31] As a biomarker of oxidative DNA damage, 8-OHdG levels directly reflect the average rate of oxidative DNA damage.[32] Daily cumulative Cr(VI) exposure has a significant correlation with urinary 8-OHdG levels after adjusting for covariates in workers.[24 31 33-35] In our study, the concentration of urinary 8-OHdG in the exposed group is significantly higher than that in the unexposed group (Table2, Table3). This result is consistent with previous studies focusing on occupational exposure, which indicates that environmental hexavalent chromium exposure induces the formation of reactive oxygen species and causes oxidative tissue and DNA damage.[36] In its turn, oxidative DNA damage can lead to consequences including cell death, mutation, and malignant transformation.[37] Some studies have shown that the concentration of 8-OHdG mainly correlates with the hexavalent chromium concentration in the air.[31 34] Therefore, the higher concentration of urinary 8-OHdG in the exposed people may be on account of higher air chromium levels. However, this relation needs further research and evidence. In the stratified analysis, we find that the level of 8-OHdG regulated with urinary creatinine in the elderly group is higher than in the younger group. In the regression model, age is a significant variant. There is a positive correlation between age and concentration of urinary 8-OHdG. A study has shown that a highly significant rise in DNA damage level can be observed in leukocyte DNA in the elderly population (mean age 67 years) and middle age group (mean age 50 years) in comparison with adults (mean age 31years).[38] The current findings are consistent with ours. The reason that the DNA damage increases with age may be a deficiency in the ability to remove the damage or the intensification of processes responsible for the damage formation, or both.[38] Some other factors may have effects on the concentration of 8-OHdG, such as smoking and alcohol. A positive correlation between the 8-OHdG levels and smoking has been observed.[39] A Danish group also found that the 8-OHdG level in urinary samples of smokers was 50%

higher as compared to nonsmokers.[40] Another study provides evidence that ethanol can induce oxidative DNA damage in human peripheral lymphocytes in vitro.[41] In young alcohol drinkers, the drinking group shows signs of increased oxidative damage compared to the non-drinking group.[42] In this study, we found that in both exposed and unexposed group, smokers or drinkers exhibited a higher concentration of 8-OHdG than non-smoking or non-drinking groups. This is also consistent with previous studies done on this issue.

In response to oxidative stress and lipid peroxidation, the antioxidant mechanisms are activated. We select GSH-Px, SOD and CAT as the three parameters to assess the antioxidant mechanisms in response to hexavalent chromium. Many researchers have shown that the activity of SOD is higher in occupational exposed groups or trivalent chromium exposed populations,[25 26 43] while some have shown that the activity of SOD and GSH-Px is decreased in the occupational-exposed group.[24] GSH-Px catalyzes the reduction of hydrogen peroxide to water and of organic hydro peroxides to less toxicity by using reduced glutathione, and the by-product, oxidized glutathione, is converted to reduced glutathione via the action of glutathione reductase using Nicotinamide Adenine Dinucleotide Phosphate (NADPH) as the electron donor.[44 45] An experiment on cells mentions that GSH-Px and CAT act in a compensatory manner to overcome oxidative stress. CAT accomplishes the basic defense at the late stages of cell growth, and GSH-Px has much higher affinity to $H_2O_2$ than catalase at low substrate concentration.[46] In our study, we find that the activity of CAT and GSH-Px is higher in the exposed group than that in the unexposed group, which means the antioxidant system is activated. However, the activity of SOD is found to have decreased in the exposed group. Antioxidants play a protective role against free radical induced damage. Therefore, their induction can be understood as a response to oxidative stress. However, if the exposure remains, the antioxidant function can be damaged during or after the exposure.[13 24 46] A decrease in the serum activity of SOD may be a sign of the impairment of the antioxidant system in this study. If the antioxidant systems are not able to reduce the ROS produced, then oxidative stress and oxidative damage may occur, leading to organism disorder, even in some cases to diseases.[17]

In the stratified analysis of oxidative parameters according to exposure status, an effect modification is found between pollution and smoking, also between pollution and alcohol use in the activity of GSH-Px. Table4 shows that the increase of the activity of GSH-Px between the unexposed group and exposed group is greater among nonsmokers and nondrinkers than among smokers and drinkers. In the exposed group, the activity of GSH-Px among nonsmokers and nondrinkers is higher than that among smokers and drinkers. However, in the unexposed group, the activity of GSH-Px among nonsmokers and nondrinkers is

lower than among smokers and drinkers. Alcohol and alcoholic beverage consumption are able to increase oxidative stress in the lungs of ethanol-fed mice.[47] Rats exposed to ethanol show a decrease in GSH-Px-1 activity and increase of hepatic glutathione reductase activities in the liver.[45] Exposure to cigarette smoke could increase intercellular ROS, and total glutathione decreased dramatically in human gingival fibroblasts.[48] In different cigarette smoke exposed rat models, the oxidative stress of the exposed group may be evaluated in different degrees.[49] In the unexposed group, smoke and alcohol use may cause the activity of GSH-Px elevating. However, in the exposed group, along with the exposure, the decrease of the activity of GSH-Px may be due to the damage of the antioxidant system. We present participants' disease status (shown in the Appendix C) and conduct stratified analysis according to disease status (shown in the Appendix D), however, we do not see any effects on the primary results.

In the analysis of relationship between duration of residence and oxidative status, we find that urine 8-OHdG concentration and the serum CAT activity have dose-response relationship with residence years in the subgroup of first exposure before 18 years old, and the serum GSH-Px activity in the subgroup of first exposure after 18 years old has a positive correlation with duration of residence. These results may indicate that longer exposure to hexavalent chromium can aggravate DNA damage and activate antioxidant response in the hexavalent chromium exposed populations. In vitro experiment, cells exposed to hexavalent chromium can activate and impair the antioxidant system with the increase of exposure time.[46] In some epidemiology studies, the researchers find that different duration of residence may have an effect on the oxidative stress levels exposed to heavy metal in women immigrants.[50] A cohort study of chromate production workers indicates that exposure duration is a important explanatory variable to the increase of lung cancer risk.[51] Our study reveals that long-term exposure to hexavalent chromium can continuously increase the health risk.

The main limitation of our study is that individual-exposed data is not obtained. This may lead to the major problems which is that we couldn't relate internal exposure to oxidative parameters. We will keep working on this project, trying to get more data to give a further clarification of the relationship between the health effects and hexavalent chromium contamination. In addition, the homogeneity of the exposed and unexposed groups is not satisfactory on account of the difference between the whole populations of the two area. For this reason, we used a multiple regression model and stratified analysis to adjust for possible confounders. Also we did not take people's nutritional status into consideration, mainly because people living in the studied areas all belong to the rural areas of Liaoning province , their diet and living habits are basically the same.

In conclusion, our research demonstrated that people living around the alloy industrial plant are at higher risk for health hazards. After adjusting for potential confounders, the results show there is an elevated oxidative stress and oxidative damage in the population exposed to hexavalent chromium compared to the population unexposed. Moreover, the effect modification presented in the stratified analysis may indicate that the combination of both hexavalent chromium and alcohol or both hexavalent chromium and smoking may cause damage to the antioxidant system. In addition, longer residence exposed to pollutants would increase people's oxidative levels.

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**Competing interests**

The authors declare that they have no competing interests.

**Authors' contributions**

Jing Xu analyzed and interpreted the data and was a major contributor in writing the manuscript. Meiduo Zhao, Lu Pei and Ruiming Zhang participanted in the field work and experiment work. Xiaolin Liu, Lanping Wei prepared all the things relating to the field work. Mingan Yang was in charge of polishing the manuscript and conducting some statistical analysis. Qun Xu designed the study and arranged the field work. All authors read and approved the final manuscript.

**Data sharing statement**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Figure Legend**

Figure 1 shows the position of six villages we conduct a survey in. The exposed district was comprised of Jinchangbao village, Nverhe village and Qiantanghe village, and the unexposed district was comprised of Baolin village, Yijiayu village, and Shifobao village. All the exposed villages were along the contaminated river, less than 10 kilometers away from the plant. The unexposed villages were at least 50 kilometers away from the plant.

**References**

1. Kim HS, Kim YJ, Seo YR. An Overview of Carcinogenic Heavy Metal: Molecular Toxicity Mechanism and Prevention. *Journal of Cancer Prevention* 2015;20(4):232-40. doi: 10.15430/jcp.2015.20.4.232

2. Qu X, Ren Z, Zhang M, et al. Sediment heavy metals and benthic diversities in Hun-Tai River, northeast of China. *Environ Sci Pollut Res Int* 2017 doi: 10.1007/s11356-017-8642-0 [published Online First: 2017/03/12]

3. Mazurek R, Kowalska J, Gasiorek M, et al. Assessment of heavy metals contamination in surface layers of Roztocze National Park forest soils (SE Poland) by indices of pollution. *Chemosphere* 2017;168:839-50. doi: 10.1016/j.chemosphere.2016.10.126 [published Online First: 2016/11/11]

4. Navarrete IA, Gabiana CC, Dumo JR, et al. Heavy metal concentrations in soils and vegetation in urban areas of Quezon City, Philippines. *Environmental monitoring and assessment* 2017;189(4):145. doi: 10.1007/s10661-017-5849-y [published Online First: 2017/03/08]

5. OLIVER MA. Soil and human health a review. *European Journal of Soil Science* 1997; 48:573-92.

6. Toxicguide for chromium. *US Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry* 2012

7. Linos A, Petralias A, Christophi CA, et al. Oral ingestion of hexavalent chromium through drinking water and cancer mortality in an industrial area of Greece--an ecological study. *Environ Health* 2011;10:50. doi: 10.1186/1476-069X-10-50

8. Kerger BD, Butler WJ, Paustenbach DJ, et al. Cancer mortality in chinese populations surrounding an alloy plant with chromium smelting operations. *J Toxicol Environ Health A* 2009;72(5):329-44. doi: 10.1080/15287390802529898

9. Chromium, nickel and welding. *IARC monographs on the evaluation of carcinogenic risks to humans* 1990;49:1-648. [published Online First: 1990/01/01]

10. Welling R, Beaumont JJ, Petersen SJ, et al. Chromium VI and stomach cancer: a meta-analysis of the current epidemiological evidence. *Occup Environ Med* 2015;72(2):151-9. doi: 10.1136/oemed-2014-102178

11. Beaumont JJ, Sedman RM, Reynolds SD, et al. Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. *Epidemiology* 2008;19(1):12-23. doi: 10.1097/EDE.0b013e31815cea4c

12. M. Valko HM, M.T.D. Cronin. Metals, Toxicity and Oxidative Stress. *Current Medicinal Chemistry* 2005;12:1161-208.

13. Patlolla AK, Barnes C, Yedjou C, et al. Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. *Environmental toxicology* 2009;24(1):66-73. doi: 10.1002/tox.20395 [published Online First: 2008/05/30]

14. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology*

2011;283(2-3):65-87. doi: 10.1016/j.tox.2011.03.001

15. Sharma B, Singh S, Siddiqi NJ. Biomedical implications of heavy metals induced imbalances in redox systems. *Biomed Res Int* 2014;2014:640754. doi: 10.1155/2014/640754

16. Dalle-Donne I, Rossi R, Colombo R, et al. Biomarkers of oxidative damage in human disease. *Clinical chemistry* 2006;52(4):601-23. doi: 10.1373/clinchem.2005.061408 [published Online First: 2006/02/18]

17. Sharma N. Free Radicals, Antioxidants and Disease. *Biology and Medicine* 2014;06(03) doi: 10.4172/0974-8369.1000214

18. Kaur R, Kaur J, Mahajan J, et al. Oxidative stress--implications, source and its prevention. *Environ Sci Pollut Res Int* 2014;21(3):1599-613. doi: 10.1007/s11356-013-2251-3 [published Online First: 2013/10/31]

19. GB 7467-87. Water quality-Determination of chrommium( Ⅵ )-1.5 Diphenylcarbohydrazide spectrophotometric method. *Ministry of Environmental Protection of the People's Republic of China*1987

20. HJ 491-2009. Soil quality-Determination of total chromium -Flame atomic absorption spectrometry. *Ministry of Environmental Protection of the People's Republic of China* 2009

21. HJ 777-2015. Ambient air and waste gas from stationary sources emission-Determination of metal elements in ambient particle matter-Inductively coupled plasma optical emission spectrometry. *Ministry of Environmental Protection of the People's Republic of China* 2015

22. Junaid M, Hashmi MZ, Malik RN, et al. Toxicity and oxidative stress induced by chromium in workers exposed from different occupational settings around the globe: A review. *Environ Sci Pollut Res Int* 2016 doi: 10.1007/s11356-016-7463-x

23. EDWIN N.FRANKEL WEV. Formation of Malonaldehyde from Lipid Oxidation Products. *Biochmicu et Biophvsicu Acta* 1983;754:264-70.

24. Wang TC, Song YS, Wang H, et al. Oxidative DNA damage and global DNA hypomethylation are related to folate deficiency in chromate manufacturing workers. *J Hazard Mater* 2012;213-214:440-6. doi: 10.1016/j.jhazmat.2012.02.024

25. Khan FH, Ambreen K, Fatima G, et al. Assessment of health risks with reference to oxidative stress and DNA damage in chromium exposed population. *Sci Total Environ* 2012;430:68-74. doi: 10.1016/j.scitotenv.2012.04.063

26. Ambreen K, Khan FH, Bhadauria S, et al. Genotoxicity and oxidative stress in chromium-exposed tannery workers in North India. *Toxicol Ind Health* 2014;30(5):405-14. doi: 10.1177/0748233712457447

27. Bagchi D, Vuchetich PJ, Bagchi M, et al. Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. *Free radical biology & medicine*

1997;22(3):471-8. [published Online First: 1997/01/01]

28. Megson IL, Haw SJ, Newby DE, et al. Association between exposure to environmental tobacco smoke and biomarkers of oxidative stress among patients hospitalised with acute myocardial infarction. *PLoS One* 2013;8(12):e81209. doi: 10.1371/journal.pone.0081209 [published Online First: 2013/12/18]
29. McCaskill ML, Kharbanda KK, Tuma DJ, et al. Hybrid malondialdehyde and acetaldehyde protein adducts form in the lungs of mice exposed to alcohol and cigarette smoke. *Alcoholism, clinical and experimental research* 2011;35(6):1106-13. doi: 10.1111/j.1530-0277.2011.01443.x [published Online First: 2011/03/25]
30. Bloomer RJ, Fisher-Wellman KH. Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. *Gender medicine* 2008;5(3):218-28. doi: 10.1016/j.genm.2008.07.002 [published Online First: 2008/08/30]
31. Kuo HW, Chang SF, Wu KY, et al. Chromium (VI) induced oxidative damage to DNA: increase of urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) among electroplating workers. *Occup Environ Med* 2003;60(8):590-4. [published Online First: 2003/07/29]
32. Floyd RA. The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis* 1990;11(9):1447-50. [published Online First: 1990/09/01]
33. Pan CH, Jeng HA, Lai CH. Biomarkers of oxidative stress in electroplating workers exposed to hexavalent chromium. *Journal of exposure science & environmental epidemiology* 2017 doi: 10.1038/jes.2016.85 [published Online First: 2017/01/26]
34. Li P, Gu Y, Yu S, et al. Assessing the suitability of 8-OHdG and micronuclei as genotoxic biomarkers in chromate-exposed workers: a cross-sectional study. *BMJ Open* 2014;4(10):e005979. doi: 10.1136/bmjopen-2014-005979
35. Ni W, Huang Y, Wang X, et al. Associations of neonatal lead, cadmium, chromium and nickel co-exposure with DNA oxidative damage in an electronic waste recycling town. *Sci Total Environ* 2014;472:354-62. doi: 10.1016/j.scitotenv.2013.11.032
36. Bagchi D, Hassoun EA, Bagchi M, et al. Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production, and generation of reactive oxygen species in Sprague-Dawley rats. *Comparative biochemistry and physiology Part C, Pharmacology, toxicology & endocrinology* 1995;110(2):177-87. [published Online First: 1995/02/01]
37. Wise SS, Holmes AL, Wise JP, Sr. Hexavalent chromium-induced DNA damage and repair mechanisms. *Reviews on environmental health* 2008;23(1):39-57. [published Online First: 2008/06/19]

38. Olinski R, Siomek A, Rozalski R, et al. Oxidative damage to DNA and antioxidant status in aging and age-related diseases. *Acta biochimica Polonica* 2007;54(1):11-26. [published Online First: 2007/01/16]

39. Kasai H. Analysis of a form of oxidative DNA damage,8-hydroxy-2X-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutation Research* 1997 387:147-63.

40. Steffen Loft KV, Marianne Ewertz,Anne Tj0nneland, Kim Overvad,Henrik Enghusen Poulsen. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Cardnogeoesis* 1992;13 (12):2241-47.

41. Yan Y, Yang JY, Mou YH, et al. Possible metabolic pathways of ethanol responsible for oxidative DNA damage in human peripheral lymphocytes. *Alcoholism, clinical and experimental research* 2011;35(1):1-9. doi: 10.1111/j.1530-0277.2010.01316.x [published Online First: 2010/10/21]

42. Rendon-Ramirez A, Cortes-Couto M, Martinez-Rizo AB, et al. Oxidative damage in young alcohol drinkers: A preliminary study. *Alcohol (Fayetteville, NY)* 2013;47(7):501-4. doi: 10.1016/j.alcohol.2013.08.002 [published Online First: 2013/10/02]

43. Junaid M, Hashmi MZ, Malik RN. Evaluating levels and health risk of heavy metals in exposed workers from surgical instrument manufacturing industries of Sialkot, Pakistan. *Environ Sci Pollut Res Int* 2016 doi: 10.1007/s11356-016-6849-0

44. Gromadzinska J, Wasowicz W, Sklodowska M, et al. The influence of atmospheric chromium on selenium content and glutathione peroxidase activity in blood of tannery workers. *Environ Health Perspect* 1996;104(12):1312-6. [published Online First: 1996/12/01]

45. Bailey SM, Patel VB, Young TA, et al. Chronic ethanol consumption alters the glutathione/glutathione peroxidase-1 system and protein oxidation status in rat liver. *Alcoholism, clinical and experimental research* 2001;25(5):726-33. [published Online First: 2001/05/24]

46. Asatiani N, Kartvelishvili T, Abuladze M, et al. Chromium (VI) can activate and impair antioxidant defense system. *Biological trace element research* 2011;142(3):388-97. doi: 10.1007/s12011-010-8806-y [published Online First: 2010/09/03]

47. Kaphalia L, Boroumand N, Hyunsu J, et al. Ethanol metabolism, oxidative stress, and endoplasmic reticulum stress responses in the lungs of hepatic alcohol dehydrogenase deficient deer mice after chronic ethanol feeding. *Toxicol Appl Pharmacol* 2014;277(2):109-17. doi: 10.1016/j.taap.2014.02.018 [published Online First: 2014/03/15]

48. Colombo G, Dalle-Donne I, Orioli M, et al. Oxidative damage in human gingival fibroblasts exposed to cigarette smoke. *Free radical biology & medicine* 2012;52(9):1584-96. doi:

10.1016/j.freeradbiomed.2012.02.030 [published Online First: 2012/03/06]

49. Ignatowicz E, Wozniak A, Kulza M, et al. Exposure to alcohol and tobacco smoke causes oxidative stress in rats. *Pharmacological reports : PR* 2013;65(4):906-13. [published Online First: 2013/10/23]

50. Wu WT, Wu CC, Lin YJ, et al. Changing blood lead levels and oxidative stress with duration of residence among Taiwan immigrants. *Journal of immigrant and minority health* 2013;15(6):1048-56. doi: 10.1007/s10903-013-9820-x [published Online First: 2013/04/06]

51. Gibb H, Hoffman H, Haver C. Biologic Implications from an Epidemiologic Study of Chromate Production Workers. The Open Epidemiology Journal2011.

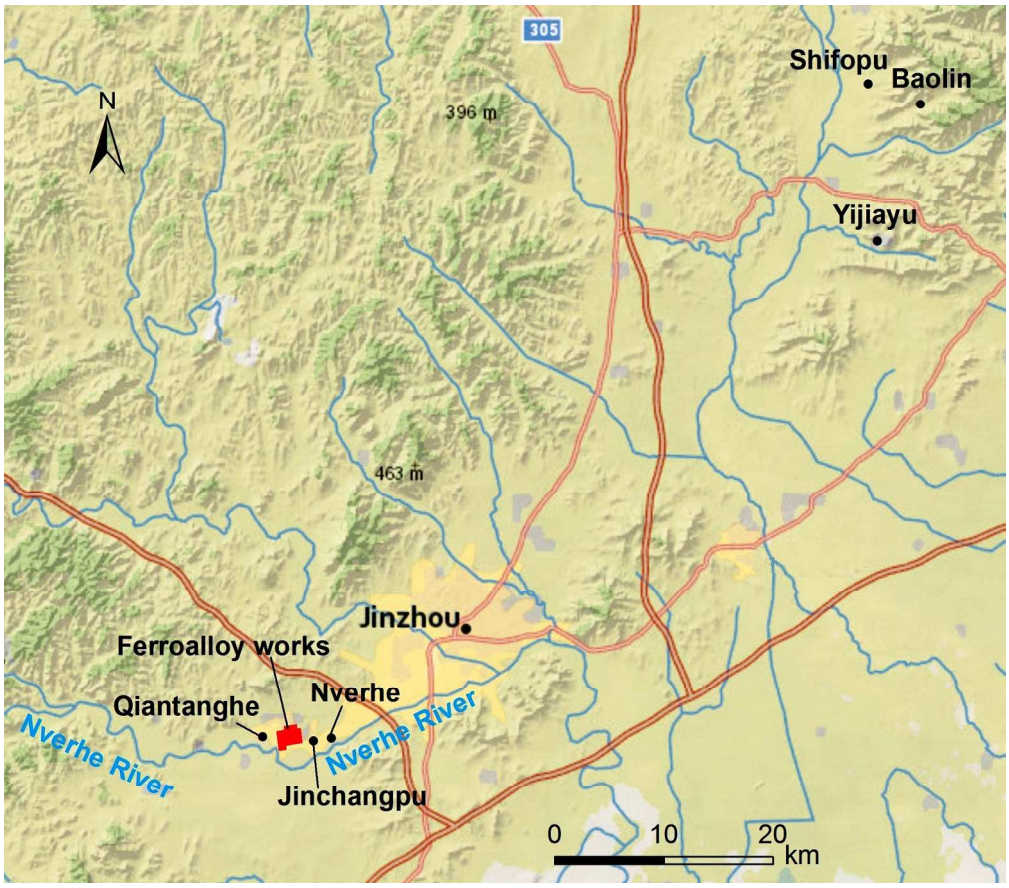


Figure 1.Locations of Exposed Areas, Unexposed Areas and Ferrochromium Factory

184x162mm (300 x 300 DPI)

# Appendix

**Appendix A. The estimate and p value of all variables in the regression Model 1**

| | | MDA (nmol/ml) | | SOD(U/ml) | | CAT(U/mL) | | GSH-Px(U/mL) | | Lg(8-OHdG)(μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β | p value | β | p value | β | p value | β | p value | β | p value |
| Age | ＜60 | ref | | ref | | ref | | ref | | ref | |
| | ⩾60 | -0.14 | 0.089 | 1.65 | 0.160 | -0.255 | 0.186 | 6.30 | 0.273 | 0.12 | ＜0.001 |
| Sex | male | ref | | ref | | ref | | ref | | ref | |
| | female | -0.22 | 0.014 | 1.96 | 0.135 | -0.71 | 0.001 | -6.62 | 0.298 | 0.06 | 0.086 |
| Exposure status | | ref | | ref | | ref | | ref | | ref | |
| unexposed | | | | | | | | | | | |
| | exposed | 0.33 | <0.001 | -14.92 | <0.001 | 1.55 | <0.001 | 43.69 | <0.001 | 0.08 | 0.012 |

**Appendix B. The estimate and p value of all variables in the regression of Model 2**

| | | MDA (nmol/m) | | SOD (U/ml) | | CAT (U/mL) | | GSH-Px (U/mL) | | Lg(8-OHdG) (μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β | p value | β | p value | β | p value | β | p value | β | p value |
| Age | < 60 | ref | | ref | | ref | | ref | | ref | |
| | ≥60 | -0.11 | 0.208 | 2.01 | 0.108 | -0.279 | 0.175 | 3.96 | 0.517 | 0.11 | 0.002 |
| Sex | male | ref | | ref | | ref | | ref | | ref | |
| | female | -0.18 | 0.111 | 2.43 | 0.133 | -0.95 | <0.001 | -13.97 | 0.076 | 0.10 | 0.025 |
| Smoking status | no | ref | | ref | | ref | | ref | | ref | |
| | yes | -0.01 | 0.895 | -0.10 | 0.948 | -0.08 | 0.758 | -14.61 | 0.049 | 0.01 | 0.791 |
| Alcohol drinking | no | ref | | ref | | ref | | ref | | ref | |
| | yes | 0.08 | 0.552 | 1.19 | 0.537 | -0.47 | 0.136 | -2.76 | 0.771 | 0.08 | 0.170 |
| Education | Primary or lower | ref | | ref | | ref | | ref | | ref | |
| | Middle or higher | 0.02 | 0.825 | 0.72 | 0.568 | -0.27 | 0.189 | -11.16 | 0.071 | -0.018 | 0.617 |
| Personal income | <2000 | ref | | ref | | ref | | ref | | ref | |
| | 2000-5000 | 0.15 | 0.150 | -0.36 | 0.810 | 0.20 | 0.424 | 2.02 | 0.781 | -0.017 | 0.693 |
| | >5000 | 0.12 | 0.211 | 0.11 | 0.940 | 0.20 | 0.4.5 | -2.84 | 0.687 | -0.032 | 0.430 |
| Exposure status | unexposed | ref | | ref | | ref | | ref | | ref | |
| | exposed | 0.32 | <0.001 | -14.93 | <0.001 | 1.60 | <0.001 | 45.66 | <0.001 | 0.093 | 0.0075 |

**Appendix C. The prevalence of diabetes and hypertension in exposed and unexposed areas**

| Variables | All (n=626) | Hexavalent chromium | | *P* value[2] |
|---|---|---|---|---|
| | | Exposure | None-exposure | |
| No. of subjects | 620 | 315 | 305 | |
| Diabetes [n (%)] | | | | 0.0284 |
| No | 545 ( 87.90 ) | 268 ( 85.08 ) | 277 ( 90.82 ) | |
| Yes | 75 ( 12.10 ) | 47 ( 14.92 ) | 28 ( 9.18 ) | |
| Hypertension [n (%)] | | | | 0.5358 |
| No | 348 ( 56.13 ) | 173 ( 54.92 ) | 175( 57.38 ) | |
| Yes | 272 ( 43.87 ) | 142 ( 45.08 ) | 130 ( 42.62 ) | |

**Appendix D. Oxidative parameters according to exposure status in different disease status**

| | | MDA(nmol/ml) | | SOD(U/ml) | | CAT(U/mL) | | GSH-Px(U/mL) | | Lg(8-OHdG)(μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed |
| Diabetes | no | 3.59±0.07** | 3.28±0.06 | 54.05±1.04** | 68.68±0.96 | 4.81±0.19** | 3.33±0.16 | 195.86±5.11 | 149.10±4.71 | 0.13±0.03* | 0.04±0.03 |
| | | (260) | (275) | ( 263 ) | (275) | (265) | (275) | (254) | (273) | (238) | (234) |
| | yes | 3.86±0.21 | 3.50±0.28 | 51.52±3.32** | 69.38±4.40 | 5.52±0.59* | 3.12±0.78 | 191.08±15.55** | 173.46±20.88 | 0.08±0.08 | -0.12±0.11 |
| | | (46) | (28) | (47) | (27) | (47) | (28) | (45) | (28) | (42) | (25) |
| Hypertension | no | 3.64±0.09** | 3.25±0.08 | 53.88±1.25** | 69.11±1.17 | 5.07±0.21** | 3.36±0.20 | 192.43±6.50** | 151.13±5.98 | 0.05±0.04 | 0.02±0.04 |
| | | (166) | (173) | (171) | (174) | (171) | (173) | (162) | (172) | (148) | (144) |
| | yes | 3.62±0.10* | 3.34±0.10 | 53.21±1.57** | 67.71±1.57 | 4.78±0.26** | 3.25±0.26 | 199.45±7.28** | 151.98±7.32 | 0.16±0.04* | 0.01±0.04 |
| | | (140) | (130) | (139) | (130) | (141) | (130) | (137) | (129) | (132) | (115) |

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# Oxidative Stress and DNA Damage in a Long-Term Hexavalent Chromium-Exposed Population in North China: A Cross-sectional Study

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# Oxidative Stress and DNA Damage in a Long-Term Hexavalent Chromium-Exposed Population in North China: A Cross-sectional Study

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**Objectives**

The International Agency for Research on Cancer (IARC) classifies hexavalent chromium [Cr(VI)] as a carcinogen. Cancer mortality is reported higher in the hexavalent chromium contaminated regions. Scientists have recommended studying the health impact of living in contaminated regions. This study aims to evaluate the health risk that populations living in hexavalent chromium contaminated areas confront.

**Design**

We conducted a cross-sectional study in rural areas in northern China. Malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were used as oxidative stress parameters and 8-hydroxy-2 deoxy guanosine (8-OHdG) as a DNA damage parameter. We collected each subject's information about socio-demographic characteristics, lifestyle and duration of residence by a questionnaire in local villages. Biological samples and environmental samples were collected the same day as the survey. We used t test, chi-square test, and linear regression analysis for univariate and multivariate analysis.

**Participants**

The sample included 319 exposed and 307 unexposed people. 447 females and 179 males were enrolled for this study. These participants met the following criteria: a) living in this district more than 10 years; b) adults older than 18 years; c) no occupational chromium exposure.

**Results**

Our study revealed that the levels of MDA ($p<0.001$), CAT ($p<0.001$), GSH-Px ($p<0.001$) and urinary 8-OHdG ($p=0.008$) in the exposed group were significantly higher than those in the unexposed group. However, SOD level was significantly lower in the exposed group compared with the unexposed group ($p<0.001$). There was an interaction effect between serum GSH-Px activity and smoking, and between serum GSH-Px activity and alcohol ($p<0.05$). Longer residence in the exposed area could increase the oxidative levels ($p<0.05$).

**Conclusions**

Findings of this study show elevated oxidative stress and DNA damage among persons exposed to hexavalent chromium.

**Key word**: hexavalent chromium, oxidative stress, oxidative damage, DNA damage, duration of residence.

**Strengths and limitations of this study:** 1.To the authors' best knowledge, this is the first paper to study the relationship between hexavalent chromium exposure and oxidative levels in non-occupational hexavalent chromium exposed populations.

2. Health survey and environment chromium hexavalent surveillance are conducted at the same temporal-spatial circumstance and the results indicate the environment contamination continuously exists in the historical polluted areas. Besides, we collect individual demographic characteristics and life style data to minimize the effect of possible confounders.

3. The main limitation of our study is that individual-exposed data are not obtained.

4. In addition, the demographic characteristics' homogeneity of the exposed and unexposed groups is not satisfactory in accounting for the difference between the whole populations of the two areas.

## 1 Introduction

Hexavalent chromium [Cr(VI)] compounds are commonly found in industrial settings such as chromite ore mining, pigment production, leather tanning, manufacture of wood preservatives, and from anticorrosive agents in cooking products.[1] Heavy metals from anthropogenic sources can be transported in the air, deposited on the soil surface and then penetrate into the water.[2 3] High concentrations of heavy metals in soil may correlate with a high concentration in plants.[4] People living near the contaminated areas may be confronted with health risks due to the heavy metal concentrations in food or water.[5] The general population may be exposed to Cr through contaminated water, food or air.[6]

Cr (VI) is considered as a carcinogen according to the IARC monographs on the evaluation of carcinogenic risks to humans.[7-10] Based on numerous occupational epidemiology studies, inhalation of Cr(VI) correlates with increased lung cancer risk.[9] Numerous epidemiologic studies report an increased risk of cancer morbidity, especially for gastrointestinal cancer, in populations exposed to Cr (VI).[7 11] For this reason, Cr (VI) contamination may pose a serious threat to population health.

The toxicity of Cr (VI) and carcinogenicity is possibly related to increased oxidative stress.[12] When Cr (VI) is reduced to a lower oxidative state, many reactive oxygen species (ROS) are formed. Therefore, one of the most important negative effects caused by extraneous Cr (VI) is the formation of ROS during the reduction of Cr (VI) in cells.[13] The generated hydroxyl radicals are

able to react with DNA bases. For this reason, the best described substance is 8-hydroxyguanosine (8-OH-dG), a good marker for oxidative damage in an organism.[14] The reduction of the extra ROS can be accomplished through enzymatic reactions and non-enzymatic reactions. Oxidative stress results from an imbalance between the production of free radicals and the antioxidant defense system, leading to a reduced capacity to detoxify free radicals and repair damage.[15] The attack of free radicals on cellular components has been studied in various pathological conditions such as in cardiovascular disease and cancer.[16-18]

Due to the industrial expansion of the mid-20$^{th}$ century, the western suburban regions of Jinzhou city have been polluted by Cr (VI).[8] Multiple studies have shown that occupational exposure to Cr (VI) induces changes in the levels of oxidative stress and oxidative damage. However, there is only a limited amount of human data on the environmental exposure of Cr (VI) in terms of oxidative stress and oxidative damage. People living close to an alloy plant could be exposed to Cr (VI) not only by the respiratory route, but also through the digestive route and skin contact. Mortality rates of stomach cancer and lung cancer in regions where water was contaminated by Cr (VI) are much higher in comparison with the regions without water contamination.[7 11] All the Cr (VI)-polluted areas in this study are along the Nver River, which have been polluted by alloy plants. Previous studies have shown that the highest chromium concentration in wells in this area were 20mg/L.[11] A ferrochromium factory was established in 1960 and since then the population living near the factory has been exposed to Cr (VI). After a long time exposure, a series of health risks may be induced in these populations. Therefore, this research mainly aims to study whether exposure to Cr (VI) could induce changes in oxidative stress and oxidative damage levels.

## 2 Materials and Methods

### 2.1 study design and population

We conducted a cross-sectional study in the villages of Jinzhou city located in the Liaoning province, to evaluate the levels of oxidative stress and oxidative damage caused by Cr (VI). We enrolled 447 females and 179 males for this study. These participants met the following criteria: a) living in this district more than 10 years; b) adults older than 18 years; c) no occupational chromium exposure. Subjects were divided into exposed and unexposed groups based on geographical position, historical data and environmental chromium level. The exposed district

was comprised of Jinchangbao village, Nverhe village and Qiantanghe village, and the unexposed district was comprised of Baolin village, Yijiayu village, and Shifobao village. Figure 1 shows the location of study areas. This map was generated by overlapping our investigated features with ArcGIS Online base map which was publicly available. This figure was produced by ArcGIS 10.2 version. All the exposed villages were along the contaminated river, less than 10 kilometers away from the plant, with a high chromium levels in the environment. Unexposed villages were at least 50 kilometers away from the plant, with a relatively low chromium levels in the environment (Figure 1, Table 1). All individuals enrolled in this study signed the informed consent form. Ethical clearance for the conduct of the study was obtained from the ethical committee, Institute of Basic Medical Sciences Chinese Academy of Medical Sciences.

**2.2 Questionnaire survey**

Specially trained postgraduate and graduate students were in charge of the participants' structured questionnaire presented face-to-face. The questionnaire was designed to collect information about socio-demographics (sex, birth date, survey date, education level, occupation status, personal income, marital status and duration of residence), lifestyle (e.g. smoking status, alcohol drinking) and whether the participant had occupational exposure to Cr(VI) and other related issues.

**2.3 Blood and urine sample collection**

Blood samples were collected after overnight fasting. Serum samples were collected into non-anticoagulation tubes and obtained by centrifugation at 3500 rpm for 10 minutes to precipitate the cellular components from the blood specimen. 10mL early morning urine samples were collected into bacteria-free centrifuge tubes. The sample was transported in an ice box to guarantee quality of the samples. Subsequently, all the experiments were conducted within 24 hours, and the remaining samples were stored at −80°C for later analysis.

**2.4 MDA concentration in serum, serum CAT, SOD, GSH-Px activity measurements**

MDA concentration in serum, serum CAT activity, serum SOD activity and serum GSH-Px activity were determined by Malondialdehyde (MDA) assay kit (TBA method), Catalase (CAT) assay kit (Visible light), Total Superoxide Dismutase (T-SOD) assay kit (Hydroxylamine method), and Glutathione Peroxidase (GSH-Px) assay kit (Colorimetric method), respectively. The Nanjing Jiancheng Bioengineering Institute (China) supplied all above kits. The absorbance of MDA and SOD for each sample was measured with a Tecan sunrise Microplate Reader (Tecan, Switzerland),

and the absorbance of CAT and GSH-Px for each sample was measured with 723 spectrophotometer (V-5100, Metash, Shanghai).

**2.5 Urinary 8-OHdG assay**

The concentration of 8-OHdG was chosen to determine the content of urinary 8-OHdG using an ELISA kit (JaAICA, Japan, 8-OHdG EIA kit) and measured with a Tecan sunrise Microplate Reader (Tecan, Switzerland). To minimize the influence of urinary density difference among subjects, the 8-OHdG level was regulated with urinary creatinine (Cre). The concentration of urinary Cre was determined by ELISA assay with a commercial kit from Roche Pharmaceutical Ltd. (Switzerland) and measured with a Roche P800 automatic analyzer (Roche Pharmaceutical Ltd, Switzerland).

**2.6 The collection and testing of environmental samples**

Environmental samples were collected in the studied area at the same time when conducting the survey. We collected groundwater samples in 7 or 8 meters-deep wells in the yard of the participants' house and soil samples in the fields' surface soil. Air samples were collected 5 days, 24 hours a day in three exposed villages and three unexposed villages with changing the sample film every 24 hours. All samples were stored in a refrigerator at 4 °C for further analysis in the laboratory. The concentration of Cr (VI) in groundwater was tested by diphenyl carbazide spectrophotometric method with the detection limit of 0.004mg/L.[19] If the detection result was under the limit, we used half of the detection limit as outcome for the statistic analysis. The total chromium level in soil and air was tested by atomic absorption spectrophotometry and Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES).[20 21]

**2.7 Quality control**

All the investigators would explain the exact meaning of each question to the subjects given that most of the subjects did not have a high education level. During the investigation, investigators would check the integrity of the questionnaire and make sure that the blood and urine samples were collected. To ensure that every individual had a unique identification to match their questionnaire and biological samples, a standard coding rule was used. The laboratory staff followed the protocol of the kit strictly to conduct analysis. The absorbance of a sample was measured three times, using the average as the final value. If the fluctuation was more than 50 percent among the three absorbance, the sample analysis was re-conducted. The

testing of the environmental samples were strictly followed the protocol of the method.

**2.8 Statistical analysis**

Epidata 3.1 was used to input the original data collected from questionnaires with the rule of double-entry and logistical error check in order to ensure accuracy. Summary statistics were provided for both categorical variables (proportion) and continuous variables. The unpaired t test was used to compare two mean values and chi-square test was used to compare categorical variables. If the data did not obey normal distribution, Wilcoxon rank sum test was used.

Subsequently, multiple general linear regression analysis was performed to analyze the main factors that affected the levels of oxidative stress and oxidative damage. To deal with skewed data, we used the log-transformation on the variable 8-OHdG in our analysis. The multivariate model did not use occupation and marital status because these two variables had less than 10% of cases in a group. All the expected and observed cases of variables used in the multivariate model was more than 5 in every bivariate cell of the 2-way tables.

To further explore the relation between hexavalent chromium exposure and oxidative stress and oxidative damage, we conducted stratified analysis according to age, sex, smoking status, alcohol drinking and education level considering the heterogeneity between the two groups. We also did stratified analysis according to disease status because some diseases may effect oxidative levels. Meanwhile, interaction terms of grouping variable and stratifying variables were added into the models to explore potential interactions. Since age at first exposure and period of residence may have an effect on the oxidative levels, we conducted a stratified analysis in the subgroup of first exposure before and after 18 years old to explore the relationship between the duration of residence and oxidative levels in exposure group. Statistical significance was defined as $p < 0.05$ (two tail). All analyses were done using SAS (version 9.4, Cary, NC, USA).

**2.9 Patients and Public Involvement**

Patients and Public were not involved in this study.

**3 Results**

Table 1 presents the chromium level in groundwater, soil and air. Cr(VI) is not detected in all the groundwater samples of unexposed area, while the maximum concentration in the samples of exposed area reached 2.5mg/L, and concentration of groundwater Cr(VI) is significantly different between the two areas (p=0.0017). The total Cr concentration in soil and air of exposed

area are both significantly higher than unexposed area with p values of <0.001 and 0.015.

**Table 1.Cr level in study area in 2016**

| Cr concentrations | Exposed area | | Unexposed area | | *p* value* |
|---|---|---|---|---|---|
| | n | Median ( Min, Q1 , Q3 , Max ) | n | Median ( Min, Q1 , Q3 , Max ) | |
| Groundwater (mg/L)† | 13 | 0.002 ( 0.002 , 0.002, 1.1 , 2.5 ) | 18 | 0.002 ( 0.002 , 0.002, 0.002 , 0.002 ) | 0.0017 |
| Soil (mg/kg) | 45 | 69.5 ( 48.7 , 59.1 , 93.9 , 417.1 ) | 30 | 29.2 ( 20.1 , 26.4 , 30.4 , 41.11 ) | <0.001 |
| Air (ng/m$^3$) | 15 | 19.3 ( 10.1 , 13.7 , 28.4 , 82.9 ) | 15 | 13.12 ( 5.0 , 10.9 , 16.8 , 18.7 ) | 0.015 |

† Hexavalent chromium concentrations

* Wilcoxon rank test was used to compare exposure and non-exposure group.

Table 2 presents the demographic characteristics of the exposed and unexposed samples. A total of 626 participants including 319 exposed villagers and 307 unexposed villagers are recruited in this study. Table 2 shows there is no significant difference in occupation, marital status, and personal income between exposed and unexposed groups. However, significant group differences (p<0.05) are found with respect to age, sex, education level, smoking status and alcohol drinking. Specifically, subjects in the exposed group are older, more likely to be female, more likely to have higher education, and less likely to smoke or drink.

Multiple regression analysis shows that serum MDA concentration (p=0.0001), serum CAT activity (p<0.0001), serum GSH-Px activity (p<0.0001) and concentration of urinary 8-OHdG (p=0.0117) are significantly higher in the exposed group compared to the unexposed group after adjusting for gender and age (Table 3, Model 1). After further adjustment for smoking status, alcohol drinking, personal income, and education level, results remain significant for serum MDA concentration (p=0.0001), serum CAT activity (p<0.0001), serum GSH-Px activity(p<0.0001) and concentration of urinary 8-OHdG (p=0.0075) (Table 3, Model 2). Multiple regression analysis shows that serum SOD activity is significantly (p<0.0001) lower in the exposed group than the unexposed group after adjusting for sex and age (Table 3, Model 1), and remains significantly lower after further adjustment for smoking, alcohol, personal income, and education (p<0.0001) (Table 3, Model 2). The serum concentration of urinary 8-OHdG between different ages is also significant in Model 1 and Model 2 with p values of <0.001 and 0.002 (data shown in the Appendix A and Appendix B).

In a stratified analysis, the difference of serum MDA concentration between exposed and unexposed groups is significant in subgroups categorized by age, sex, smoking status, alcohol drinking and education level (Table 4). Serum CAT activity shows significant differences between exposed and unexposed groups by age, sex, smoking status, alcohol drinking and education (Table 4). The stratified analysis also shows that the difference of serum SOD activity between exposed and unexposed groups is significant in all of the subgroup analyses by age, sex, smoking status, alcohol drinking and education. The difference of serum GSH-Px activity between exposed and unexposed groups is significant by age, sex, smoking status, alcohol drinking and education. An interaction effect is observed between serum GSH-Px activity and smoking status, and between GSH-Px activity and alcohol drinking (Table 4). As for the urine 8-OHdG, the stratified analysis indicates a significant difference between exposed and unexposed groups in age, sex, smoking status, and alcohol drinking (Table 4). The mean and standard deviation of participants' duration of residence in exposure group is 45 years and 13 years. Duration of residence has a positive association with the oxidative levels (Table 5). The serum CAT activity (p=0.0466) and the urine 8-OHdG concentration (p=0.0242) become higher with increase of resident years in the subgroup of first exposure age before 18. Besides, the serum GSH-Px activity (p=0.0369) is higher along with the increase of resident years in the subgroup of first exposure after 18.

**Table 2. Demographic characteristics and oxidative parameters of the study participants.**

| Variables | All | Hexavalent chromium | | *p* value* |
|---|---|---|---|---|
| | (n=626) | Exposure | None-exposure | |
| No. of subjects | 626 | 319 | 307 | |
| Age (y) [1] | 60.34 ± 10.57 | 61.21 ± 9.36 | 59.44 ± 11.64 | 0.0377 |
| Sex [n (%)] | | | | <0.0001 |
| Male | 179 ( 28.59 ) | 69 ( 21.63 ) | 110 ( 35.83 ) | |
| Female | 447 ( 71.41 ) | 250 ( 78.37 ) | 197 ( 64.17 ) | |
| Education [n (%)] | | | | <0.0001 |
| Primary school or lower | 342 ( 54.63 ) | 146 ( 45.77 ) | 196 ( 63.84 ) | |
| Middle school or higher | 283 ( 45.21 ) | 173 ( 54.23 ) | 110 ( 35.83 ) | |
| Occupation [n (%)] | | | | 0.0665 |
| Peasant | 589 ( 94.09 ) | 295 ( 92.48 ) | 294 ( 95.77 ) | |
| Others | 37 ( 6.91 ) | 24 ( 7.52 ) | 13 ( 4.23 ) | |
| Smoking status [n (%)] | | | | 0.0049 |
| No | 458 ( 73.16 ) | 249 ( 75.86 ) | 209 ( 68.08 ) | |
| Yes | 168 ( 26.84 ) | 70 ( 21.94 ) | 98 ( 31.27 ) | |
| Alcohol drinking [n (%)] | | | | 0.0033 |
| No | 511 ( 81.63 ) | 275 ( 86.21 ) | 236 ( 77.85 ) | |
| Yes | 114 ( 18.21 ) | 44 ( 13.79 ) | 70 ( 22.80 ) | |
| Marital status [n (%)] | | | | 0.5424 |
| Married | 580 ( 92.65 ) | 298 ( 93.42 ) | 282 ( 91.86 ) | |
| Others | 45 ( 7.19 ) | 21 ( 6.58 ) | 24 ( 7.82 ) | |
| Personal income (yuan)[n ( %)] | | | | 0.9049 |
| <2000 | 285 ( 45.53 ) | 148 ( 46.39 ) | 137 ( 44.63 ) | |
| 2000-5000 | 152 ( 24.28 ) | 76 ( 23.82 ) | 76 ( 24.76 ) | |
| >5000 | 189 ( 30.19 ) | 95 ( 29.78 ) | 94 ( 30.62 ) | |

*A Student's t test was used for continuous variables, and a chi-square test was used for categorical variables.

**Table 3. Multiple factors regression analysis on oxidative parameters and chromium in exposure and non-exposure**

| Parameter | Model | exposure (Lsmean ± SE) | Non-exposure (Lsmean ± SE) | β‡ | *p* value |
|---|---|---|---|---|---|
| MDA (nmol-mL$^{-1}$) | model1* | 3.62 ± 0.06 | 3.29 ± 0.06 | 0.33 | 0.0001 |
| | model2※ | 3.65 ± 0.07 | 3.33 ± 0.06 | 0.32 | 0.0001 |
| SOD (U-mL$^{-1}$) | model1 | 53.87 ± 0.90 | 68.80 ± 0.85 | -14.92 | <0.0001 |
| | model2 | 54.06 ± 1.02 | 68.99 ± 0.95 | -14.93 | <0.0001 |
| GSH-Px (U-mL$^{-1}$) | model1 | 197.47 ± 4.44 | 153.77 ± 4.12 | 43.69 | <0.0001 |
| | model2 | 194.99 ± 5.00 | 149.33 ± 4.64 | 45.66 | <0.0001 |
| CAT (U-mL$^{-1}$) | model1 | 4.86 ± 0.15 | 3.31 ± 0.14 | 1.55 | <0.0001 |
| | model2 | 4.77 ± 0.17 | 3.17 ± 0.16 | 1.60 | <0.0001 |
| 8-OHdG) †(ng/μmol-Cre) | model1 | 0.11 ± 0.03 | 0.02 ± 0.02 | 0.08 | 0.0117 |
| | model2 | 0.12 ± 0.03 | 0.03 ± 0.03 | 0.09 | 0.0075 |

*model1 is adjusted for sex, age ※model2 is adjusted for sex, age, personal income, education, smoking and alcohol use. And p value of every model is less than 0.05. †logarithm-transformed to adjust the distribution ‡beta coefficient of the exposure status using unexposed as reference

**Table 4.Oxidative parameters average level according to exposure status in subgroups (Lsmeans±SE(n))**

| | | MDA(nmol/ml) | | SOD(U/ml) | | CAT(U/mL) | | GSH-Px(U/mL) | | Lg(8-OHdG)(μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed |
| Age | <60 | 3.79±0.11** | 3.44±0.10 | 52.44±1.59** | 68.84±1.43 | 4.82±0.28** | 3.28±0.25 | 189.47±7.87** | 150.49±7.00 | 0.08±0.04* | -0.02±0.04 |
| | | (134) | (152) | (138) | (152) | (138) | (152) | (131) | (152) | (127) | (135) |
| | ≥60 | 3.53±0.09** | 3.25±0.09 | 54.68±1.29** | 68.42±1.36 | 4.55±0.21** | 2.93±0.21 | 200.54±6.61** | 145.41±6.56 | 0.19±0.04 | 0.10±0.04 |
| | | (172) | (151) | (173) | (151) | (174) | (151) | (169) | (149) | (154) | (126) |
| Sex | male | 3.68±0.12 | 3.50±0.10 | 53.99±1.63** | 67.65±1.30 | 5.54±0.33** | 3.46±0.26 | 196.89±8.92* | 161.25±7.11 | 0.13±0.04* | -0.05±0.04 |
| | | (68) | (109) | (68) | (109) | (68) | (109) | (67) | (108) | (63) | (96) |
| | female | 3.46±0.13** | 3.12±0.13 | 55.78±1.98** | 71.17±1.98 | 4.49±0.30** | 3.04±0.30 | 189.16±9.63** | 138.98±9.43 | 0.10±0.04 | 0.04±0.04 |
| | | (238) | (194) | (243) | (194) | (244) | (194) | (233) | (193) | (218) | (165) |
| Smoking | no | 3.74±0.10** | 3.35±0.10 | 53.48±1.52** | 68.95±1.49 | 4.94±0.25** | 3.24±0.25 | 205.03±7.29**† | 148.22±7.08 | 0.10±0.04* | -0.02±0.04 |
| | | (238) | (207) | (242) | (207) | (243) | (207) | (234) | (206) | (221) | (177) |
| | yes | 3.50±0.13 | 3.40±0.11 | 55.46±1.75** | 68.84±1.50 | 4.70±0.28** | 3.29±0.24 | 172.10±9.25 | 155.54±7.77 | 0.09±0.05 | 0.07±0.05 |
| | | (68) | (95) | (69) | (96) | (69) | (96) | (66) | (95) | (60) | (84) |
| Alcohol | no | 3.62±0.08** | 3.25±0.08 | 53.20±1.24** | 68.74±1.21 | 5.07±0.21** | 3.46±0.20 | 198.38±5.90**※ | 146.20±5.70 | 0.07±0.04* | -0.02±0.03 |
| | | (263) | (234) | (268) | (234) | (269) | (234) | (260) | (233) | (240) | (201) |
| | yes | 3.40±0.19 | 3.34±0.16 | 56.95±2.47** | 68.56±2.18 | 4.73±0.37** | 3.17±0.33 | 175.01±13.78 | 157.48±11.84 | 0.12±0.07 | 0.04±0.06 |
| | | (43) | (69) | (43) | (69) | (43) | (69) | (40) | (68) | (41) | (60) |
| Education | Primary or lower | 3.53±0.11 | 3.31±0.09 | 53.07±1.51** | 69.16±1.26 | 4.89±0.24** | 3.40±0.20 | 199.52±6.98** | 151.10±5.82 | 0.14±0.05 | 0.05±0.05 |
| | | (139) | (194) | (142) | (194) | (142) | (194) | (141) | (193) | (126) | (165) |
| | Middle or higher | 3.76±0.09** | 3.28±0.10 | 54.82±1.43** | 68.32±1.59 | 4.59±0.24** | 2.87±0.27 | 192.31±7.51** | 150.22±8.16 | 0.10±0.03 | 0.02±0.04 |
| | | (167) | (109) | (169) | (109) | (170) | (109) | (159) | (108) | (155) | (96) |

*p<0.05 versus unexposed population **p<0.001 versus unexposed population †p=0.0357 of interaction effect between smoking and exposure status ※p=0.006 of interaction effect between alcohol and exposure status

**Table 5.Analysis duration of residence (years) and oxidative parameters by different age at first exposure in the exposure group (Lsmeans±SE)**

| Age at first exposure(years) | GSH-Px※(U-mL-1) | | | | CAT※(U-mL-1) | | | | 8-OHdG†※(ng/μmol-Cre) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Residence less than 38 years | Residence between 38 and 55 years | Residence more than 55 years | *P* value* | Residence less than 38 years | Residence between 38 and 55 years | Residence more than 55 years | *P* value * | Residence less than 38 years | Residence between 38 and 55 years | Residence more than 55 years | *P* value * |
| ≤18 | 180.14 ± 38.01 | 167.46 ± 15.12 | 180.94 ± 11.32 | 0.6902 | 3.07 ± 1.04 | 4.23 ± 0.44 | 4.94 ± 0.32 | 0.0466 | -0.22 ± 0.15 | 0.10 ± 0.06 | 0.16 ± 0.05 | 0.0242 |
| >18 | 196.73 ± 10.61 | 202.27 ± 16.42 | 257.21 ± 10.14 | 0.0369 | 5.00 ± 0.48 | 5.10 ± 0.73 | 5.67 ± 0.89 | 0.5725 | 0.10 ± 0.05 | 0.12 ± 0.08 | 0.17 ± 0.05 | 0.5417 |

*p for trend †logarithm-transformed to adjust the distribution ※The model is adjusted for age, sex, personal income, education, smoking status and alcohol use

## 4 Discussion

Because the environmental Cr concentration (underground water, soil and air) is fairly high in exposed regions, people living in the exposed regions are generally at high risk to be exposed to Cr (VI). Since the 1970s, the villagers in the exposed area stopped using the groundwater as drinking water thanks to the government's water improvement project, while people living in the villages still use the groundwater to irrigate fields and do some washing. Moreover, they would contact the high chromium concentration soil with their hands and skin when cultivating. Thus, villagers in exposed area may contact Cr (VI) through skin or hand-to-mouth route. [5] The Cr in air provides a respiration way for exposed villagers to contact higher Cr concentration. [8] Therefore, through all above exposure pathways, people living in the Cr (VI)-exposed area may have a high risk to be exposed to Cr compared to those in unexposed area.

In this study, our results indicate that after adjusting possible confounders, people living in Cr (VI) exposed areas have higher lipids and DNA damage levels than those in unexposed areas. In addition, Cr (VI) exposure affects the antioxidant system, such as the activation of the antioxidant system or damaging the antioxidant system.[17 22] Besides significant differences between the exposed and unexposed group, additional factors affect the results, such as sex, age, smoking and alcohol use. Moreover, longer residence in exposed areas may increase the health risk.

Lipid peroxidation has been suggested to play a key role in many biological processes, and MDA has long been used as a marker for secondary products of lipid peroxidation.[23] Many studies have shown a significant increase of MDA in Cr exposed groups compared to unexposed groups both in humans and animals, which may indicate that Cr exposure results in depletion of the antioxidant defense elements, subsequently causing lipid peroxidation.[24-27] In our study, we find that the MDA concentration of the exposed group is significantly higher than that in the unexposed group after adjusting for sex and age or even in the full model. The evaluated MDA concentration indicates increased rate of oxidative stress levels in the lipid in exposed populations. However, results of the stratified analysis show that the MDA concentration is not significantly affected by exposure to Cr (VI) in the subgroup that smokes or consumes alcohol. On the other hand, exposure to Cr (VI) still affects the non-smoking population and those who do not consume alcohol. Many studies have shown that after adjusting for potential confounders, smoking and drinking can elevate the concentration of MDA in both animal models and in humans.[28 29] However, we do not find significant association either between smoking and MDA concentration or between alcohol and MDA concentration. In our view, this lack of correlation could be mainly due to the strong

influence of Cr (VI), which covers the effect that smoking and alcohol have on the MDA concentration. There have been some discrepancies in the concentration of MDA in gender in previous studies. In trivalent chromium exposed populations, the MDA concentration is higher in females than that in males.[25] However, in normal populations, concentration of MDA is lower in females than that in males.[30] In this study, the MDA concentration in females is lower than that in males and is consistent in both the exposed and unexposed groups. This is consistent with results in studies done on normal populations.

Reactive oxygen species form when Cr (VI) reduces to a lower oxidation state, and the free radicals may attack DNA thereby disrupting cellular functions and integrity.[27] Thus DNA damage produces alterations in DNA, strand breaks and DNA-protein crosslinks. 8-OHdG is a major oxidative adducts formed by radicals inducing damage to DNA.[31] As a biomarker of oxidative DNA damage, 8-OHdG levels directly reflect the average rate of oxidative DNA damage.[32] Daily cumulative Cr (VI) exposure has a significant correlation with urinary 8-OHdG levels after adjusting for covariates in workers.[24 31 33-35]

In our study, the concentration of urinary 8-OHdG in the exposed group is significantly higher than in the unexposed group (Table 3). This is consistent with previous studies focusing on occupational exposure, which indicates that environmental Cr (VI) exposure induces the formation of reactive oxygen species and causes oxidative tissue and DNA damage.[36] In its turn, oxidative DNA damage can lead to consequences including cell death, mutation, and malignant transformation.[37] Some studies have shown that the concentration of 8-OHdG mainly correlates with the Cr (VI) concentration in the air.[31 34] Therefore, the higher concentration of urinary 8-OHdG in the exposed people may be on account of higher air chromium levels. However, this relation needs further research and evidence.

In the stratified analysis, we find that the level of 8-OHdG regulated with urinary creatinine in the elderly group is higher than that in the younger group. In the regression model, age is a significant variant. There is a positive correlation between age and concentration of urinary 8-OHdG. A study has shown that a highly significant rise in DNA damage level can be observed in leukocyte DNA in the elderly population (mean age 67 years) and middle age group (mean age 50 years) in comparison with adults (mean age 31 years).[38] These findings are consistent with ours. The reason that the DNA damage increases with age may be a deficiency in the ability to remove the damage or the intensification of processes responsible for the damage formation, or both.[38] Some other factors may have effects on the concentration of 8-OHdG, such as smoking and alcohol. A positive correlation between the 8-OHdG levels and smoking has been observed.[39] A Danish group also finds that the 8-OHdG level in urinary samples of smokers is 50% higher as compared to nonsmokers.[40] Another study provides evidence that ethanol can induce oxidative DNA

damage in human peripheral lymphocytes in vitro.[41] In young alcohol drinkers, the drinking group shows signs of increased oxidative damage compared to the non-drinking group.[42] In this study, we find that in both exposed and unexposed group, smokers or drinkers exhibited a higher concentration of 8-OHdG than non-smoking or non-drinking groups. This is also consistent with previous studies done on this issue.

In response to oxidative stress and lipid peroxidation, the antioxidant mechanisms are activated. We selected GSH-Px, SOD and CAT as the three parameters to assess the antioxidant mechanisms in response to Cr (VI). Many researchers have shown that the activity of SOD is higher in occupational exposed groups or trivalent chromium exposed populations,[25 26 43] while some have shown that the activity of SOD and GSH-Px is decreased in the occupational-exposed group.[24] GSH-Px catalyzes the reduction of hydrogen peroxide to water and of organic hydro peroxides to less toxicity by using reduced glutathione, and the by-product, oxidized glutathione, is converted to reduced glutathione via the action of glutathione reductase using Nicotinamide Adenine Dinucleotide Phosphate (NADPH) as the electron donor.[44 45] An experiment on cells mentions that GSH-Px and CAT act in a compensatory manner to overcome oxidative stress. CAT accomplishes the basic defense at the late stages of cell growth, and GSH-Px has much higher affinity to $H_2O_2$ than catalase at low substrate concentration.[46]

In our study, we find that the activity of CAT and GSH-Px is higher in the exposed group than that in the unexposed group, which means the antioxidant system is activated. However, the activity of SOD is found to have decreased in the exposed group. Antioxidants play a protective role against free radical induced damage. Therefore, their induction can be understood as a response to oxidative stress. However, if the exposure remains, the antioxidant function can be damaged during or after the exposure.[13 24 46] A decrease in the serum activity of SOD may be a sign of the impairment of the antioxidant system in this study. If the antioxidant systems are not able to reduce the ROS produced, then oxidative stress and oxidative damage may occur, leading to organism disorder, even in some cases to diseases.[17]

In the stratified analysis of oxidative parameters according to exposure status, an effect modification is found between pollution and smoking, also between pollution and alcohol use in the activity of GSH-Px. In the exposed group, the activity of GSH-Px among nonsmokers and nondrinkers is higher than that among smokers and drinkers. However, in the unexposed group, the activity of GSH-Px among nonsmokers and nondrinkers is lower than that among smokers and drinkers. Alcohol and alcoholic beverage consumption are able to increase oxidative stress in the lungs of ethanol-fed mice.[47] Rats exposed to ethanol show a decrease in GSH-Px-1 activity and increase of hepatic glutathione reductase activities in the liver.[45] Exposure to cigarette smoke could increase intercellular ROS, and total glutathione

decreases dramatically in human gingival fibroblasts.[48] In different cigarette smoke exposed rat models, the oxidative stress of the exposed group may be evaluated in different degrees.[49] In the unexposed group, smoke and alcohol use may cause the activity of GSH-Px elevating. However, in the exposed group, along with the exposure, the decrease of the activity of GSH-Px may be due to the damage of the antioxidant system. We present participants' disease status (shown in the Appendix C) and conduct stratified analysis according to disease status (shown in the Appendix D), however, we do not see any effects on the primary results.

In the analysis of relationship between duration of residence and oxidative status, we find that urine 8-OHdG concentration and the serum CAT activity have dose-response relationship with residence years in the subgroup of first exposure before 18 years old, and the serum GSH-Px activity in the subgroup of first exposure after 18 years old has a positive correlation with duration of residence. These results may indicate that longer exposure to hexavalent chromium can aggravate DNA damage and activate antioxidant response in the hexavalent chromium exposed populations. In vitro experiment, cells exposed to hexavalent chromium can activate and impair the antioxidant system with the increase of exposure time.[46] In some epidemiology studies, the researchers find that different duration of residence may have an effect on the oxidative stress levels exposed to heavy metal in women immigrants.[50] A cohort study of chromate production workers indicates that exposure duration is an important explanatory variable to the increase of lung cancer risk.[51] Our study reveals that long-term exposure to Cr (VI) can continuously increase the health risk.

The main limitation of our study is that individual-exposure data is not obtained. This may lead to the major problems which is that we could not relate internal exposure to oxidative parameters. We will keep working on this project, trying to get more data to give a further clarification of the relationship between the health effects and hexavalent chromium contamination. In addition, the homogeneity of the exposed and unexposed groups is not satisfactory in accounting for the difference between the whole populations of the two areas. For this reason, we used a multiple regression model and stratified analysis to adjust for possible confounders. Also we did not take people's nutritional status into consideration, mainly because people living in the studied areas all belong to the rural areas of Liaoning province , their diet and living habits are basically the same.

In conclusion, our research demonstrates that people living around the alloy industrial plant are at higher risk for health hazards. After adjusting for potential confounders, the results show elevated oxidative stress and oxidative damage in the population exposed to Cr (VI) compared to the unexposed

population. Moreover, the effect modification presented in the stratified analysis may indicate that the combination of both Cr (VI) and alcohol or both Cr (VI) and smoking may cause damage to antioxidant system. In addition, longer residence exposed to pollutants would increase people's oxidative levels.

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**Competing interests**

The authors declare that they have no competing interests.

**Authors' contributions**

Jing Xu analyzed and interpreted the data and was a major contributor in writing the manuscript. Meiduo Zhao, Lu Pei and Ruiming Zhang participanted in the field work and experiment work. Xiaolin Liu, Lanping Wei prepared all the things relating to the field work. Mingan Yang was in charge of polishing the manuscript and conducting some statistical analysis. Qun Xu designed the study and arranged the field work. All authors read and approved the final manuscript.

**Data sharing statement**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Figure Legend**

Figure 1 shows the position of six villages where we conduct a survey. The exposed district was comprised of Jinchangbao village, Nverhe village and Qiantanghe village, and the unexposed district was comprised of Baolin village, Yijiayu village, and Shifobao village. All the exposed villages were along the contaminated river, less than 10 kilometers away from the plant. The unexposed villages were at least 50 kilometers away from the plant. This map was generated by overlapping our investigated features with ArcGIS Online base map which was publicly available. This figure was produced by ArcGIS 10.2 version.

**References**

1. Kim HS, Kim YJ, Seo YR. An Overview of Carcinogenic Heavy Metal: Molecular Toxicity Mechanism and Prevention. *Journal of Cancer Prevention* 2015;20(4):232-40. doi: 10.15430/jcp.2015.20.4.232

2. Qu X, Ren Z, Zhang M, et al. Sediment heavy metals and benthic diversities in Hun-Tai River, northeast of China. *Environ Sci Pollut Res Int* 2017 doi: 10.1007/s11356-017-8642-0 [published Online First: 2017/03/12]

3. Mazurek R, Kowalska J, Gasiorek M, et al. Assessment of heavy metals contamination in surface layers of Roztocze National Park forest soils (SE Poland) by indices of pollution. *Chemosphere* 2017;168:839-50. doi: 10.1016/j.chemosphere.2016.10.126 [published Online First: 2016/11/11]

4. Navarrete IA, Gabiana CC, Dumo JR, et al. Heavy metal concentrations in soils and vegetation in urban areas of Quezon City, Philippines. *Environmental monitoring and assessment* 2017;189(4):145. doi: 10.1007/s10661-017-5849-y [published Online First: 2017/03/08]

5. OLIVER MA. Soil and human health a review. *European Journal of Soil Science* 1997; 48:573-92.

6. Toxicguide for chromium. *US Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry* 2012

7. Linos A, Petralias A, Christophi CA, et al. Oral ingestion of hexavalent chromium through drinking water and cancer mortality in an industrial area of Greece--an ecological study. *Environ Health* 2011;10:50. doi: 10.1186/1476-069X-10-50

8. Kerger BD, Butler WJ, Paustenbach DJ, et al. Cancer mortality in chinese populations surrounding an alloy plant with chromium smelting operations. *J Toxicol Environ Health A* 2009;72(5):329-44. doi: 10.1080/15287390802529898

9. Chromium, nickel and welding. *IARC monographs on the evaluation of carcinogenic risks to humans* 1990;49:1-648. [published Online First: 1990/01/01]

10. Welling R, Beaumont JJ, Petersen SJ, et al. Chromium VI and stomach cancer: a meta-analysis of the current epidemiological evidence. *Occup Environ Med* 2015;72(2):151-9. doi: 10.1136/oemed-2014-102178

11. Beaumont JJ, Sedman RM, Reynolds SD, et al. Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. *Epidemiology* 2008;19(1):12-23. doi: 10.1097/EDE.0b013e31815cea4c

12. M. Valko HM, M.T.D. Cronin. Metals, Toxicity and Oxidative Stress. *Current Medicinal Chemistry* 2005;12:1161-208.

13. Patlolla AK, Barnes C, Yedjou C, et al. Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. *Environmental toxicology* 2009;24(1):66-73. doi: 10.1002/tox.20395 [published Online First: 2008/05/30]

14. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology* 2011;283(2-3):65-87. doi: 10.1016/j.tox.2011.03.001

15. Sharma B, Singh S, Siddiqi NJ. Biomedical implications of heavy metals induced imbalances in redox systems. *Biomed Res Int* 2014;2014:640754. doi: 10.1155/2014/640754

16. Dalle-Donne I, Rossi R, Colombo R, et al. Biomarkers of oxidative damage in human disease. *Clinical chemistry* 2006;52(4):601-23. doi: 10.1373/clinchem.2005.061408 [published Online First: 2006/02/18]

17. Sharma N. Free Radicals, Antioxidants and Disease. *Biology and Medicine* 2014;06(03) doi: 10.4172/0974-8369.1000214

18. Kaur R, Kaur J, Mahajan J, et al. Oxidative stress--implications, source and its prevention. *Environ Sci Pollut Res Int* 2014;21(3):1599-613. doi: 10.1007/s11356-013-2251-3 [published Online First: 2013/10/31]

19. GB 7467-87. Water quality-Determination of chrommium( Ⅵ )-1.5 Diphenylcarbohydrazide spectrophotometric method. *Ministry of Environmental Protection of the People's Republic of China*1987

20. HJ 491-2009. Soil quality-Determination of total chromium -Flame atomic absorption spectrometry. *Ministry of Environmental Protection of the People's Republic of China* 2009

21. HJ 777-2015. Ambient air and waste gas from stationary sources emission-Determination of metal elements in ambient particle matter-Inductively coupled plasma optical emission spectrometry. *Ministry of Environmental Protection of the People's Republic of China* 2015

22. Junaid M, Hashmi MZ, Malik RN, et al. Toxicity and oxidative stress induced by chromium in workers exposed from different occupational settings around the globe: A review. *Environ Sci Pollut Res Int* 2016 doi: 10.1007/s11356-016-7463-x

23. EDWIN N.FRANKEL WEV. Formation of Malonaldehyde from Lipid Oxidation Products. *Biochmicu et Biophvsicu Acta* 1983;754:264-70.

24. Wang TC, Song YS, Wang H, et al. Oxidative DNA damage and global DNA hypomethylation are related to folate deficiency in chromate manufacturing workers. *J Hazard Mater* 2012;213-214:440-6. doi: 10.1016/j.jhazmat.2012.02.024

25. Khan FH, Ambreen K, Fatima G, et al. Assessment of health risks with reference to oxidative stress and DNA damage in chromium exposed population. *Sci Total Environ* 2012;430:68-74. doi: 10.1016/j.scitotenv.2012.04.063

26. Ambreen K, Khan FH, Bhadauria S, et al. Genotoxicity and oxidative stress in chromium-exposed tannery workers in North India. *Toxicol Ind Health* 2014;30(5):405-14. doi: 10.1177/0748233712457447

27. Bagchi D, Vuchetich PJ, Bagchi M, et al. Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. *Free radical biology & medicine* 1997;22(3):471-8. [published Online First: 1997/01/01]

28. Megson IL, Haw SJ, Newby DE, et al. Association between exposure to environmental tobacco smoke and biomarkers of oxidative stress among patients hospitalised with acute myocardial infarction. *PLoS One*

2013;8(12):e81209. doi: 10.1371/journal.pone.0081209 [published Online First: 2013/12/18]

29. McCaskill ML, Kharbanda KK, Tuma DJ, et al. Hybrid malondialdehyde and acetaldehyde protein adducts form in the lungs of mice exposed to alcohol and cigarette smoke. *Alcoholism, clinical and experimental research* 2011;35(6):1106-13. doi: 10.1111/j.1530-0277.2011.01443.x [published Online First: 2011/03/25]
30. Bloomer RJ, Fisher-Wellman KH. Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. *Gender medicine* 2008;5(3):218-28. doi: 10.1016/j.genm.2008.07.002 [published Online First: 2008/08/30]
31. Kuo HW, Chang SF, Wu KY, et al. Chromium (VI) induced oxidative damage to DNA: increase of urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) among electroplating workers. *Occup Environ Med* 2003;60(8):590-4. [published Online First: 2003/07/29]
32. Floyd RA. The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis* 1990;11(9):1447-50. [published Online First: 1990/09/01]
33. Pan CH, Jeng HA, Lai CH. Biomarkers of oxidative stress in electroplating workers exposed to hexavalent chromium. *Journal of exposure science & environmental epidemiology* 2017 doi: 10.1038/jes.2016.85 [published Online First: 2017/01/26]
34. Li P, Gu Y, Yu S, et al. Assessing the suitability of 8-OHdG and micronuclei as genotoxic biomarkers in chromate-exposed workers: a cross-sectional study. *BMJ Open* 2014;4(10):e005979. doi: 10.1136/bmjopen-2014-005979
35. Ni W, Huang Y, Wang X, et al. Associations of neonatal lead, cadmium, chromium and nickel co-exposure with DNA oxidative damage in an electronic waste recycling town. *Sci Total Environ* 2014;472:354-62. doi: 10.1016/j.scitotenv.2013.11.032
36. Bagchi D, Hassoun EA, Bagchi M, et al. Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production, and generation of reactive oxygen species in Sprague-Dawley rats. *Comparative biochemistry and physiology Part C, Pharmacology, toxicology & endocrinology* 1995;110(2):177-87. [published Online First: 1995/02/01]
37. Wise SS, Holmes AL, Wise JP, Sr. Hexavalent chromium-induced DNA damage and repair mechanisms. *Reviews on environmental health* 2008;23(1):39-57. [published Online First: 2008/06/19]
38. Olinski R, Siomek A, Rozalski R, et al. Oxidative damage to DNA and antioxidant status in aging and age-related diseases. *Acta biochimica Polonica* 2007;54(1):11-26. [published Online First: 2007/01/16]
39. Kasai H. Analysis of a form of oxidative DNA damage,8-hydroxy-2X-deoxyguanosine, as a marker of cellular

oxidative stress during carcinogenesis. *Mutation Research* 1997 387:147-63.

40. Steffen Loft KV, Marianne Ewertz,Anne Tj0nneland, Kim Overvad,Henrik Enghusen Poulsen. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Cardnogeoesis* 1992;13 (12):2241-47.
41. Yan Y, Yang JY, Mou YH, et al. Possible metabolic pathways of ethanol responsible for oxidative DNA damage in human peripheral lymphocytes. *Alcoholism, clinical and experimental research* 2011;35(1):1-9. doi: 10.1111/j.1530-0277.2010.01316.x [published Online First: 2010/10/21]
42. Rendon-Ramirez A, Cortes-Couto M, Martinez-Rizo AB, et al. Oxidative damage in young alcohol drinkers: A preliminary study. *Alcohol (Fayetteville, NY)* 2013;47(7):501-4. doi: 10.1016/j.alcohol.2013.08.002 [published Online First: 2013/10/02]
43. Junaid M, Hashmi MZ, Malik RN. Evaluating levels and health risk of heavy metals in exposed workers from surgical instrument manufacturing industries of Sialkot, Pakistan. *Environ Sci Pollut Res Int* 2016 doi: 10.1007/s11356-016-6849-0
44. Gromadzinska J, Wasowicz W, Sklodowska M, et al. The influence of atmospheric chromium on selenium content and glutathione peroxidase activity in blood of tannery workers. *Environ Health Perspect* 1996;104(12):1312-6. [published Online First: 1996/12/01]
45. Bailey SM, Patel VB, Young TA, et al. Chronic ethanol consumption alters the glutathione/glutathione peroxidase-1 system and protein oxidation status in rat liver. *Alcoholism, clinical and experimental research* 2001;25(5):726-33. [published Online First: 2001/05/24]
46. Asatiani N, Kartvelishvili T, Abuladze M, et al. Chromium (VI) can activate and impair antioxidant defense system. *Biological trace element research* 2011;142(3):388-97. doi: 10.1007/s12011-010-8806-y [published Online First: 2010/09/03]
47. Kaphalia L, Boroumand N, Hyunsu J, et al. Ethanol metabolism, oxidative stress, and endoplasmic reticulum stress responses in the lungs of hepatic alcohol dehydrogenase deficient deer mice after chronic ethanol feeding. *Toxicol Appl Pharmacol* 2014;277(2):109-17. doi: 10.1016/j.taap.2014.02.018 [published Online First: 2014/03/15]
48. Colombo G, Dalle-Donne I, Orioli M, et al. Oxidative damage in human gingival fibroblasts exposed to cigarette smoke. *Free radical biology & medicine* 2012;52(9):1584-96. doi: 10.1016/j.freeradbiomed.2012.02.030 [published Online First: 2012/03/06]
49. Ignatowicz E, Wozniak A, Kulza M, et al. Exposure to alcohol and tobacco smoke causes oxidative stress in rats. *Pharmacological reports : PR* 2013;65(4):906-13. [published Online First: 2013/10/23]

50. Wu WT, Wu CC, Lin YJ, et al. Changing blood lead levels and oxidative stress with duration of residence among Taiwan immigrants. *Journal of immigrant and minority health* 2013;15(6):1048-56. doi: 10.1007/s10903-013-9820-x [published Online First: 2013/04/06]

51. Gibb H, Hoffman H, Haver C. Biologic Implications from an Epidemiologic Study of Chromate Production Workers. The Open Epidemiology Journal2011.

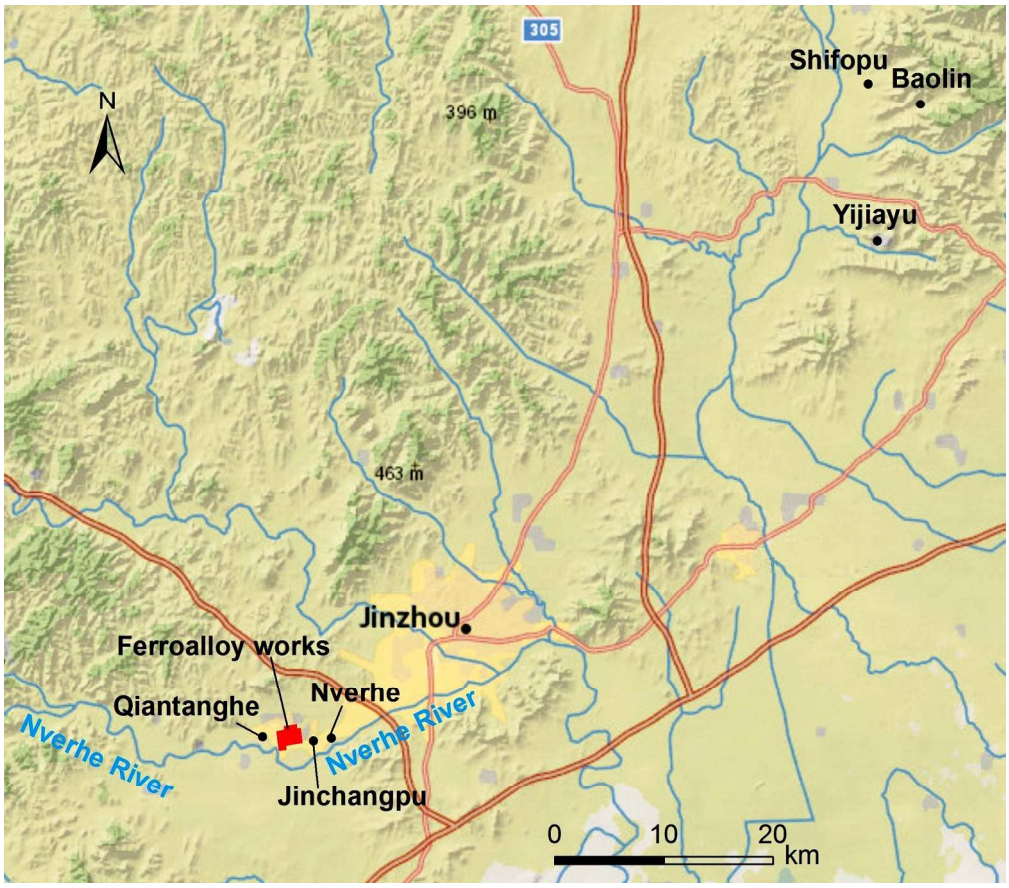


Figure 1.Locations of Exposed Areas, Unexposed Areas and Ferrochromium Factory

184x162mm (300 x 300 DPI)

# Appendix

**Appendix A. The estimate and p value of all variables in the regression Model 1**

| | | MDA (nmol/ml) | | SOD(U/ml) | | CAT(U/mL) | | GSH-Px(U/mL) | | Lg(8-OHdG)(μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β | p value | β | p value | β | p value | β | p value | β | p value |
| Age | ＜60 | ref | | ref | | ref | | ref | | ref | |
| | ⩾60 | -0.14 | 0.089 | 1.65 | 0.160 | -0.255 | 0.186 | 6.30 | 0.273 | 0.12 | ＜0.001 |
| Sex | male | ref | | ref | | ref | | ref | | ref | |
| | female | -0.22 | 0.014 | 1.96 | 0.135 | -0.71 | 0.001 | -6.62 | 0.298 | 0.06 | 0.086 |
| Exposure status | | ref | | ref | | ref | | ref | | ref | |
| unexposed | | | | | | | | | | | |
| | exposed | 0.33 | <0.001 | -14.92 | <0.001 | 1.55 | <0.001 | 43.69 | <0.001 | 0.08 | 0.012 |

**Appendix B. The estimate and p value of all variables in the regression of Model 2**

| | | MDA (nmol/m) | | SOD (U/ml) | | CAT (U/mL) | | GSH-Px (U/mL) | | Lg(8-OHdG) (μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β | p value | β | p value | β | p value | β | p value | β | p value |
| Age | < 60 | ref | | ref | | ref | | ref | | ref | |
| | ≥60 | -0.11 | 0.208 | 2.01 | 0.108 | -0.279 | 0.175 | 3.96 | 0.517 | 0.11 | 0.002 |
| Sex | male | ref | | ref | | ref | | ref | | ref | |
| | female | -0.18 | 0.111 | 2.43 | 0.133 | -0.95 | <0.001 | -13.97 | 0.076 | 0.10 | 0.025 |
| Smoking status | no | ref | | ref | | ref | | ref | | ref | |
| | yes | -0.01 | 0.895 | -0.10 | 0.948 | -0.08 | 0.758 | -14.61 | 0.049 | 0.01 | 0.791 |
| Alcohol drinking | no | ref | | ref | | ref | | ref | | ref | |
| | yes | 0.08 | 0.552 | 1.19 | 0.537 | -0.47 | 0.136 | -2.76 | 0.771 | 0.08 | 0.170 |
| Education | Primary or lower | ref | | ref | | ref | | ref | | ref | |
| | Middle or higher | 0.02 | 0.825 | 0.72 | 0.568 | -0.27 | 0.189 | -11.16 | 0.071 | -0.018 | 0.617 |
| Personal income | <2000 | ref | | ref | | ref | | ref | | ref | |
| | 2000-5000 | 0.15 | 0.150 | -0.36 | 0.810 | 0.20 | 0.424 | 2.02 | 0.781 | -0.017 | 0.693 |
| | >5000 | 0.12 | 0.211 | 0.11 | 0.940 | 0.20 | 0.4.5 | -2.84 | 0.687 | -0.032 | 0.430 |
| Exposure status | unexposed | ref | | ref | | ref | | ref | | ref | |
| | exposed | 0.32 | <0.001 | -14.93 | <0.001 | 1.60 | <0.001 | 45.66 | <0.001 | 0.093 | 0.0075 |

**Appendix C. The prevalence of diabetes and hypertension in exposed and unexposed areas**

| Variables | All (n=626) | Hexavalent chromium | | *P* value[2] |
|---|---|---|---|---|
| | | Exposure | None-exposure | |
| No. of subjects | 620 | 315 | 305 | |
| Diabetes [n (%)] | | | | 0.0284 |
| No | 545 ( 87.90 ) | 268 ( 85.08 ) | 277 ( 90.82 ) | |
| Yes | 75 ( 12.10 ) | 47 ( 14.92 ) | 28 ( 9.18 ) | |
| Hypertension [n (%)] | | | | 0.5358 |
| No | 348 ( 56.13 ) | 173 ( 54.92 ) | 175( 57.38 ) | |
| Yes | 272 ( 43.87 ) | 142 ( 45.08 ) | 130 ( 42.62 ) | |

**Appendix D. Oxidative parameters according to exposure status in different disease status**

| | | MDA(nmol/ml) | | SOD(U/ml) | | CAT(U/mL) | | GSH-Px(U/mL) | | Lg(8-OHdG)(μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed |
| Diabetes | no | 3.59±0.07** | 3.28±0.06 | 54.05±1.04** | 68.68±0.96 | 4.81±0.19** | 3.33±0.16 | 195.86±5.11 | 149.10±4.71 | 0.13±0.03* | 0.04±0.03 |
| | | (260) | (275) | ( 263 ) | (275) | (265) | (275) | (254) | (273) | (238) | (234) |
| | yes | 3.86±0.21 | 3.50±0.28 | 51.52±3.32** | 69.38±4.40 | 5.52±0.59* | 3.12±0.78 | 191.08±15.55** | 173.46±20.88 | 0.08±0.08 | -0.12±0.11 |
| | | (46) | (28) | (47) | (27) | (47) | (28) | (45) | (28) | (42) | (25) |
| Hypertension | no | 3.64±0.09** | 3.25±0.08 | 53.88±1.25** | 69.11±1.17 | 5.07±0.21** | 3.36±0.20 | 192.43±6.50** | 151.13±5.98 | 0.05±0.04 | 0.02±0.04 |
| | | (166) | (173) | (171) | (174) | (171) | (173) | (162) | (172) | (148) | (144) |
| | yes | 3.62±0.10* | 3.34±0.10 | 53.21±1.57** | 67.71±1.57 | 4.78±0.26** | 3.25±0.26 | 199.45±7.28** | 151.98±7.32 | 0.16±0.04* | 0.01±0.04 |
| | | (140) | (130) | (139) | (130) | (141) | (130) | (137) | (129) | (132) | (115) |

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# Oxidative stress and DNA damage in a long-term hexavalent chromium-exposed population in north China: A cross-sectional study

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| | |

# Oxidative stress and DNA damage in a long-term hexavalent chromium-exposed population in north China: A cross-sectional study

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**Objectives**

The International Agency for Research on Cancer (IARC) classifies hexavalent chromium [Cr(VI)] as a human carcinogen. As reported, cancer mortality was higher in the Cr(VI) contaminated areas. Scientists have recommended studying the health impact on the people living in contaminated areas. This study aims to evaluate the health risk for the people living in Cr(VI) contaminated areas.

**Design**

We conducted a cross-sectional study in rural areas of northeastern China. Malondialdehyde (MDA), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were used as oxidative stress parameters and 8-hydroxy-2 deoxy guanosine (8-OHdG) was as a DNA damage biomarker. We collected information about demographics, lifestyles and length of residence for all participants by a questionnaire. Biological specimens and environmental media samples were collected on the same day as the survey done. We used t-test, chi-square test, Wilcoxon rank-sum test, and multivariate linear regression analysis.

**Participants**

The participants in this study included 319 exposed to hexavalent chromium and 307 unexposed with 447 females and 179 males. These participants met the following criteria: a) living in the areas more than 10 years; b) age older than 18 years; and c) without occupational chromium exposure.

**Results**

Our study revealed that serum concentration of MDA ($p<0.001$), serum activities of CAT ($p<0.001$) and GSH-Px ($p<0.001$), as well as urine concentration of 8-OHdG ($p=0.008$) in the exposed group were significantly higher than in the unexposed group. However, serum SOD activity was significantly lower in the exposed group, compared with that in the unexposed ($p<0.001$). Hexavalent chromium exposure and smoking have an interaction effect on GSH-Px activity ($p<0.05$), and hexavalent chromium exposure and alcohol drinking also have an interaction effect on GSH-Px activity ($p<0.05$). Longer residence in the exposed areas increased oxidative levels ($p<0.05$).

**Conclusions**

Findings of this study showed elevated oxidative stress and DNA damage in the people exposed

to Cr(VI).

**Key words**: hexavalent chromium, oxidative stress, oxidative damage, DNA damage.

**Strengths and limitations of this study:** 1.To the authors' best knowledge, this is the first paper to study the relationship between hexavalent chromium exposure and oxidative stress levels in non-occupationally exposed people.

2. Health survey and environmental surveillance for hexavalent chromium were conducted concurrently in previously environmentally polluted areas. Besides, we collected individual demographic characteristics and life style data to minimize the possible confounding in analysis.

3. The main limitation is that individual-exposed data is not obtained.

4. In addition, demographic homogeneity of the exposed and unexposed groups was not so satisfactory.

## 1 Introduction

Hexavalent chromium [Cr(VI)] compounds are commonly found in industrial settings, such as chromite ore mining, pigment production, leather tanning, manufacture of wood preservatives, and anticorrosive process in kitchen utensils production (electroplating).[1] Heavy metals from anthropogenic sources can be transported in the air, deposited on the soil surface and then penetrate into the water.[2 3] High concentrations of heavy metals in soil may correlate with a high concentration in plants.[4] People living near the contaminated areas may be confronted with health risks due to the heavy metal concentrations in food or water.[5] The general population may be exposed to chromium (Cr) through contaminated water, food or air.[6]

Cr(VI) is considered a human carcinogen according to the IARC monographs on the evaluation of carcinogenic risks to humans.[7-10] Based on numerous studies in occupational epidemiology, inhalation of Cr(VI) correlated with increased lung cancer risk.[9] Numerous epidemiologic studies reported an increased risk of cancer morbidity, especially for gastrointestinal cancer, in populations exposed to Cr(VI).[7 11] For this reason, Cr(VI) contamination may pose a serious threat to population health.

Toxicity and carcinogenicity of Cr(VI) is possibly related to increased oxidative stress.[12] When Cr(VI) is reduced to a lower oxidative state, many reactive oxygen species (ROS) form. Therefore, one of the most important negative effects caused by extraneous Cr(VI) is the formation of ROS during the reduction of Cr(VI) in cells.[13] The generated hydroxyl radicals are able to react with

DNA bases. For this reason, the best described substance is 8-hydroxyguanosine (8-OH-dG), a good marker for oxidative damage in an organism.[14] Reduction of the extra ROS can be accomplished through enzymatic and non-enzymatic reactions. Oxidative stress results from an imbalance between the production of free radicals and the antioxidant defense system, leading to a reduced capacity to detoxify free radicals and repair damage.[15] The attack of free radicals on cellular components has been studied in various pathological conditions such as in cardiovascular diseases and cancers.[16-18] Animal experiments indicate that Cr(VI) exposure results in depletion of the antioxidant defense elements, subsequently causing lipid peroxidation.[19] Lipid peroxidation has been suggested to play a key role in many biological processes, and MDA has long been used as a marker for secondary products of lipid peroxidation.[20]

Due to the industrial expansion of the mid-20$^{th}$ century, the western suburban areas of Jinzhou city, Liaoning province, northeast China have been environmentally polluted by Cr(VI).[8] Multiple studies have shown that occupational exposure to Cr(VI) induced changes in oxidative stress and oxidative damage. However, there is only a limited amount of human data on the environmental exposure of Cr(VI) in terms of oxidative stress and oxidative damage. People living close to a ferroalloy plant could be exposed to Cr(VI) not only by respiratory route, but also through digestive and cutaneous routes. Mortality rates of stomach cancer and lung cancer in areas where water was contaminated by Cr(VI) were much higher in comparison with those in areas without contamination.[7 11] All the Cr(VI)-polluted areas in this study are along the Nver River, where has been polluted by ferroalloy factory. Previous studies have shown that the highest Cr concentration in well water of this area was 20mg/L.[11] A ferroalloy factory was established there in 1960 and since then people living near the factory have been exposed to Cr(VI). After long-time exposure, a series of health risks may be induced in them. Therefore, this study mainly aims to study whether environmental exposure to Cr(VI) can induce changes in oxidative stress and oxidative damage.

## 2 Materials and Methods

### 2.1 Study design and population

We conducted a cross-sectional study in the villages of Jinzhou city located in Liaoning province, northeast China to evaluate changes in the levels of oxidative stress and oxidative damage caused by Cr(VI). We enrolled 626 participants, 447 females and 179 males, in the study,

who met the following criteria: a) living in the areas for more than 10 years; b) age older than 18 years; and c) without occupational Cr exposure. The participants were divided into exposed and unexposed groups based on their geographical position, historical data and environmental Cr levels, with A1 village, A2 village and A3 village as exposed areas, and B1 village, B2 village, and B3 village as unexposed. Figure 1 shows a map of the study areas, which was generated by the ArcGIS Online base map publicly available and produced by the ArcGIS 10.2 software. All the exposed villages were along the contaminated river, less than 10 kilometers away from the ferroalloy factory, with high Cr levels in the environment. The unexposed villages were at least 50 kilometers away from the factory, with relatively low Cr levels in the environment (Figure 1, Table 1). All enrolled individuals signed the informed consent forms. The study was approved by the Institutional Review Board (IRB) at the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences.

**2.2 Questionnaire survey**

Specially trained under-graduate and post-graduate students were involved in face-to-face interviews for the participants with an ad hoc questionnaire, which was designed to collect information about socio-demographics (sex, birth date, survey date, education level, occupation status, personal income, marital status and length of residence), lifestyles (e.g., smoking, alcohol drinking) as well as occupational exposure to Cr(VI) and other related issues.

**2.3 Blood and urine sample collection**

Whole blood was collected into an EDTA anticoagulation tube. Serum was collected into a non-anticoagulation tube and obtained by centrifugation at 3500 rpm for 10 minutes to precipitate the cellular components. Urine specimen was collected into a bacteria-free centrifuge tube. All the samples were transported by an ice box to guarantee their quality. Subsequently, all the laboratory examinations were conducted within 24 hours, and remaining samples were stored at −80°C for later analysis.

**2.4 MDA concentration in serum, serum CAT, SOD, GSH-Px activity measurements**

Serum concentration of MDA, serum activities of CAT, SOD and GSH-Px were determined with Malondialdehyde (MDA) assay kit (TBA method), Catalase (CAT) assay kit (Visible light), Total Superoxide Dismutase (T-SOD) assay kit (Hydroxylamine method), and Glutathione Peroxidase (GSH-Px) assay kit (Colorimetric method), respectively, which were supplied by Nanjing Jiancheng Bioengineering Institute (China).

**2.5 Urinary 8-OHdG and urinary creatinine measurements**

Urine concentration of 8-OHdG was determined with an ELISA kit (JaAICA, Japan, 8-OHdG EIA kit). To minimize the influence of urine density difference among participants, the 8-OHdG concentration was regulated with urine creatinine (Cre), which was determined by ELISA with a commercial kit from Roche Pharmaceutical Ltd. (Switzerland).

**2.6 Collection and testing of environmental media samples**

Environmental media samples were collected in the studied area at the same time as the survey conducted. We collected groundwater samples from seven or eight meters-deep wells in the yards of participant house and soil samples from the field surface. Air samples were collected 24 hours a day for five days in three exposed villages and three unexposed villages, with sampling membranes changed every 24 hours. All samples were stored in refrigerators at 4 °C for further laboratory analysis. Concentration of Cr(VI) in groundwater was determined by diphenyl carbazide spectrophotometric method with a detection limit of 0.004mg/L.[21] If detection result was lower than the limit, half of the detection limit was used in statistical analysis. Total Cr level of soil and air was determined by atomic absorption spectrophotometry and Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES).[22 23]

**2.7 Quality Control**

All investigators would explain the exact meaning of each question to the participants given that most of them did not have a higher education level. After the survey, investigators would check the integrity of questionnaire completed and make sure that blood and urine specimens were collected. To ensure that every participant had a unique identification to match their questionnaires and biological specimens, a standard coding system was used. The laboratory staff were required to follow the protocol of the kit instructions strictly in conducting analysis. Absorbance for each specimen was measured three times, using the average as its final value. If its fluctuation was more than 50 percent among the three absorbance, the analysis would be re-conducted. Testing of the environmental samples was strictly conducted according to the protocol.

**2.8 Statistical Analysis**

EpiData 3.1 software was used for input of original data collected from questionnaires with double-entry and check for logic errors to ensure their accuracy. Summary statistics were

provided for both categorical (proportion) and continuous variables. The unpaired t-test was used to compare two mean values and chi-square test was used to compare categorical variables. If the data did not meet normal distribution, Wilcoxon rank-sum test was used.

Subsequently, multiple general linear regression analysis was performed to analyze the main factors that affected the levels of oxidative stress and oxidative damage. To deal with skewed data, we used log-transformation for the variable 8-OHdG in our analysis. Occupation and marital status were not used in multivariate regression analysis, because these two variables had less than 10% of cases in a group. All the expected and observed number with the variables used in the multivariate regression model had more than 5 in each cell of the 2-way tables.

To explore the relationship between Cr(VI) exposure and oxidative stress and damage, we conducted further analysis stratified by age, sex, smoking status, alcohol drinking, and education level, respectively, as well as stratified by disease status because some diseases could affect oxidative levels. Interaction terms were added into the models to explore potential interactions between variables. We conducted a stratified analysis in the subgroups of first exposure before and after 18 years old to explore the relationship between the length of residence and oxidative levels in the exposed group, since age at first exposure and length of residence may have an effect on the oxidative levels. The statistical significance of a linear trend was tested by including the median of each category as a continuous variable in the regression model. Statistical significance was defined as p value less than 0.05 (two-tailed). All analyses were done using the SAS software (version 9.4, Cary, NC, USA).

**2.9 Patients and Public Involvement**

Patients and public were not involved in this study.

**3 Results**

Table 1 presents the Cr level in groundwater, soil and air. Cr(VI) was not detected in any groundwater samples of the unexposed areas, while maximum concentration of Cr(VI) in groundwater samples in the exposed areas reached 2.5mg/L, with a significantly statistical difference (p=0.0017). The total Cr concentrations in soil and air samples from the exposed areas both are significantly higher than unexposed areas, with p-values of less than 0.001 and 0.015, respectively.

**Table 1.Cr levels in varied samples collected from the study areas, in 2016**

| Samples | Exposed areas | | Unexposed areas | | *p* value* |
|---|---|---|---|---|---|
| | N | Median ( Min, Q1 , Q3 , Max ) | N | Median ( Min, Q1 , Q3 , Max ) | |
| Groundwater (mg/L)† | 13 | 0.002 ( 0.002 , 0.002, 1.1 , 2.5 ) | 18 | 0.002 ( 0.002 , 0.002, 0.002 , 0.002 ) | 0.0017 |
| Soil (mg/kg) | 45 | 69.5 ( 48.7 , 59.1 , 93.9 , 417.1 ) | 30 | 29.2 ( 20.1 , 26.4 , 30.4 , 41.11 ) | <0.001 |
| Air (ng/m$^3$) | 15 | 19.3 ( 10.1 , 13.7 , 28.4 , 82.9 ) | 15 | 13.12 ( 5.0 , 10.9 , 16.8 , 18.7 ) | 0.015 |

† Hexavalent chromium

* Wilcoxon rank-sum test was used to compare the difference between the exposed areas and unexposed areas.

Table 2 presents demographic characteristics of the 626 participants living in the exposed and unexposed areas, 319 in the exposed villages and 307 unexposed. Table 2 shows there is no significant difference in occupation, marital status, or personal income between exposed and unexposed groups. However, significant differences ($p<0.05$) with respect to age, sex, education level, smoking status and alcohol drinking between the two groups were found. Specifically, participants in the exposed group are older, more likely to be female, more likely to have higher education level and less likely to smoke or drink than in the unexposed.

Results of multivariate regression analysis showed that serum MDA concentration ($p=0.0001$), serum CAT activity ($p<0.0001$), serum GSH-Px activity ($p<0.0001$) and urine concentration of 8-OHdG ($p=0.0117$) were significantly higher in the exposed group compared to the unexposed group adjusted for gender and age (Table 3, Model 1). After further adjustment for smoking status, alcohol drinking, personal income, and education level, results remain statistical significance for serum MDA concentration ($p=0.0001$), serum CAT activity ($p<0.0001$), serum GSH-Px activity($p<0.0001$) and urine concentration of 8-OHdG ($p=0.0075$) (Table 3, Model 2). Multivariate regression analysis showed that serum SOD activity was significantly ($p<0.0001$) lower in the exposed group than the unexposed group adjusted for sex and age (Table 3, Model 1), which remained significantly lower further adjusted for smoking status, alcohol drinking, personal income, and education level ($p<0.0001$) (Table 3, Model 2). Furthermore, urine concentration of 8-OHdG was also significantly different among varied age groups in both Model

1 and Model 2 with p-values of less than 0.001 and 0.002 (data shown in the Appendix A and Appendix B). Table 4 shows difference in serum MDA concentration between exposed and unexposed groups with analysis for subgroups categorized by age, sex, smoking status, alcohol drinking, and education level. Similar results were reported for serum activities of CAT, SOD and GSH-Px, and urine 8-OHdG concentration (Table 4). Cr(VI) exposure and smoking have an interaction effect on GSH-Px activity, and Cr(VI) exposure and alcohol drinking also have an interaction effect on GSH-Px activity (Table 4).

Length of residence positively associated with the oxidative levels (Table 5), with a mean of 45 years and standard deviation of 13 years. Both serum CAT activity (p=0.0466) and urine 8-OHdG concentration (p=0.0242) increased with length of residence in the subgroup of their first exposure at ages under 18 years, and serum GSH-Px activity (p=0.0369) also increased with the length of residence in those first exposed at ages over 18 years.

**Table 2. Demographic characteristics of the study participants.**

| Variables | All ( N = 626 ) | Hexavalent chromium | | *p* value* |
|---|---|---|---|---|
| | | Exposure ( N = 319 ) | None-exposure ( N = 307 ) | |
| Age (yrs.) [1] | 60.34 ± 10.57 | 61.21 ± 9.36 | 59.44 ± 11.64 | 0.0377 |
| Sex [n (%)] | | | | <0.0001 |
| Male | 179 ( 28.59 ) | 69 ( 21.63 ) | 110 ( 35.83 ) | |
| Female | 447 ( 71.41 ) | 250 ( 78.37 ) | 197 ( 64.17 ) | |
| Education level [n (%)] | | | | <0.0001 |
| Primary school or lower | 342 ( 54.63 ) | 146 ( 45.77 ) | 196 ( 63.84 ) | |
| Middle school or higher | 283 ( 45.21 ) | 173 ( 54.23 ) | 110 ( 35.83 ) | |
| Occupation [n (%)] | | | | 0.0665 |
| Farmer | 589 ( 94.09 ) | 295 ( 92.48 ) | 294 ( 95.77 ) | |
| Others | 37 ( 6.91 ) | 24 ( 7.52 ) | 13 ( 4.23 ) | |
| Smoking status [n (%)] | | | | 0.0049 |
| No | 458 ( 73.16 ) | 249 ( 75.86 ) | 209 ( 68.08 ) | |
| Yes | 168 ( 26.84 ) | 70 ( 21.94 ) | 98 ( 31.27 ) | |
| Alcohol drinking [n (%)] | | | | 0.0033 |
| No | 511 ( 81.63 ) | 275 ( 86.21 ) | 236 ( 77.85 ) | |
| Yes | 114 ( 18.21 ) | 44 ( 13.79 ) | 70 ( 22.80 ) | |
| Marital status [n (%)] | | | | 0.5424 |
| Married | 580 ( 92.65 ) | 298 ( 93.42 ) | 282 ( 91.86 ) | |
| Others | 45 ( 7.19 ) | 21 ( 6.58 ) | 24 ( 7.82 ) | |
| Personal income (RMB yuan) [n (%)] | | | | 0.9049 |
| <2000 | 285 ( 45.53 ) | 148 ( 46.39 ) | 137 ( 44.63 ) | |
| 2000-5000 | 152 ( 24.28 ) | 76 ( 23.82 ) | 76 ( 24.76 ) | |
| >5000 | 189 ( 30.19 ) | 95 ( 29.78 ) | 94 ( 30.62 ) | |

*A Student's t test was used for continuous variables, and a chi-square test was used for categorical variables.

**Table 3. Average oxidative parameters according to exposure and non-exposure**

| Parameter | Model | exposure (Lsmean ± SE) | Non-exposure (Lsmean ± SE) | β‡ | *p* value |
|---|---|---|---|---|---|
| MDA (nmol/mL) | model1* | 3.62 ± 0.06 | 3.29 ± 0.06 | 0.33 | 0.0001 |
| | model2※ | 3.65 ± 0.07 | 3.33 ± 0.06 | 0.32 | 0.0001 |
| SOD (U/mL) | model1 | 53.87 ± 0.90 | 68.80 ± 0.85 | -14.92 | <0.0001 |
| | model2 | 54.06 ± 1.02 | 68.99 ± 0.95 | -14.93 | <0.0001 |
| GSH-Px (U/mL) | model1 | 197.47 ± 4.44 | 153.77 ± 4.12 | 43.69 | <0.0001 |
| | model2 | 194.99 ± 5.00 | 149.33 ± 4.64 | 45.66 | <0.0001 |
| CAT (U/mL) | model1 | 4.86 ± 0.15 | 3.31 ± 0.14 | 1.55 | <0.0001 |
| | model2 | 4.77 ± 0.17 | 3.17 ± 0.16 | 1.60 | <0.0001 |
| 8-OHdG† (ng/μmol-Cre) | model1 | 0.11 ± 0.03 | 0.02 ± 0.02 | 0.08 | 0.0117 |
| | model2 | 0.12 ± 0.03 | 0.03 ± 0.03 | 0.09 | 0.0075 |

*model1 is adjusted for sex, age ※model2 is adjusted for sex, age, personal income, education, smoking and alcohol use. p-value of every model is less than 0.05. †with logarithm-transformed for normal distribution ‡beta coefficient of regression, with the unexposed as reference

**Table 4.Average levels of varied oxidative parameters in the exposed and unexposed groups by age, sex, smoking status, alcohol drink and education level [Lsmeans ± SE (n)]**

| | | MDA (nmol/mL) | | SOD (U/mL) | | CAT (U/mL) | | GSH-Px (U/mL) | | Lg (8-OHdG) (μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposure | None-exposure | Exposure | None-exposure | Exposure | None-exposure | Exposure | None-exposure | Exposure | None-exposure |
| Age | <60 | 3.79±0.11** | 3.44±0.10 | 52.44±1.59** | 68.84±1.43 | 4.82±0.28** | 3.28±0.25 | 189.47±7.87** | 150.49±7.00 | 0.08±0.04* | -0.02±0.04 |
| | | (134) | (152) | (138) | (152) | (138) | (152) | (131) | (152) | (127) | (135) |
| | ≥60 | 3.53±0.09** | 3.25±0.09 | 54.68±1.29** | 68.42±1.36 | 4.55±0.21** | 2.93±0.21 | 200.54±6.61** | 145.41±6.56 | 0.19±0.04 | 0.10±0.04 |
| | | (172) | (151) | (173) | (151) | (174) | (151) | (169) | (149) | (154) | (126) |
| Sex | male | 3.68±0.12 | 3.50±0.10 | 53.99±1.63** | 67.65±1.30 | 5.54±0.33** | 3.46±0.26 | 196.89±8.92* | 161.25±7.11 | 0.13±0.04* | -0.05±0.04 |
| | | (68) | (109) | (68) | (109) | (68) | (109) | (67) | (108) | (63) | (96) |
| | female | 3.46±0.13** | 3.12±0.13 | 55.78±1.98** | 71.17±1.98 | 4.49±0.30** | 3.04±0.30 | 189.16±9.63** | 138.98±9.43 | 0.10±0.04 | 0.04±0.04 |
| | | (238) | (194) | (243) | (194) | (244) | (194) | (233) | (193) | (218) | (165) |
| Smoking | no | 3.74±0.10** | 3.35±0.10 | 53.48±1.52** | 68.95±1.49 | 4.94±0.25** | 3.24±0.25 | 205.03±7.29**† | 148.22±7.08 | 0.10±0.04* | -0.02±0.04 |
| | | (238) | (207) | (242) | (207) | (243) | (207) | (234) | (206) | (221) | (177) |
| | yes | 3.50±0.13 | 3.40±0.11 | 55.46±1.75** | 68.84±1.50 | 4.70±0.28** | 3.29±0.24 | 172.10±9.25 | 155.54±7.77 | 0.09±0.05 | 0.07±0.05 |
| | | (68) | (95) | (69) | (96) | (69) | (96) | (66) | (95) | (60) | (84) |
| Alcohol | no | 3.62±0.08** | 3.25±0.08 | 53.20±1.24** | 68.74±1.21 | 5.07±0.21** | 3.46±0.20 | 198.38±5.90**※ | 146.20±5.70 | 0.07±0.04* | -0.02±0.03 |
| | | (263) | (234) | (268) | (234) | (269) | (234) | (260) | (233) | (240) | (201) |
| | yes | 3.40±0.19 | 3.34±0.16 | 56.95±2.47** | 68.56±2.18 | 4.73±0.37** | 3.17±0.33 | 175.01±13.78 | 157.48±11.84 | 0.12±0.07 | 0.04±0.06 |
| | | (43) | (69) | (43) | (69) | (43) | (69) | (40) | (68) | (41) | (60) |
| Education | Primary or lower | 3.53±0.11 | 3.31±0.09 | 53.07±1.51** | 69.16±1.26 | 4.89±0.24** | 3.40±0.20 | 199.52±6.98** | 151.10±5.82 | 0.14±0.05 | 0.05±0.05 |
| | | (139) | (194) | (142) | (194) | (142) | (194) | (141) | (193) | (126) | (165) |
| | Middle or higher | 3.76±0.09** | 3.28±0.10 | 54.82±1.43** | 68.32±1.59 | 4.59±0.24** | 2.87±0.27 | 192.31±7.51** | 150.22±8.16 | 0.10±0.03 | 0.02±0.04 |
| | | (167) | (109) | (169) | (109) | (170) | (109) | (159) | (108) | (155) | (96) |

*p<0.05 versus unexposed population **p<0.001 versus unexposed population †p=0.0357 of interaction effect between smoking status and exposure status ※p=0.006 of interaction effect between alcohol drinking and exposure status

**Table 5. Oxidative parameters of different residence length (years) by age at first exposure in the exposed group ( Lsmeans ± SE )**

| Age at first exposure(years) | GSH-Px※(U/mL) | | | | CAT※(U/mL) | | | | 8-OHdG†※(ng/μmol-Cre) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ≤38 years | 39–55 years | ⩾56 years | $P_{for\ trend}$* | ≤38 years | 39–55 years | ⩾56 years | $P_{for\ trend}$ | ≤38 years | 39–55 years | ⩾56 years | $P_{for\ trend}$ |
| ≤18 | 180.14 ± 38.01 | 167.46 ± 15.12 | 180.94 ± 11.32 | 0.6902 | 3.07 ± 1.04 | 4.23 ± 0.44 | 4.94 ± 0.32 | 0.0466 | -0.22 ± 0.15 | 0.10 ± 0.06 | 0.16 ± 0.05 | 0.0242 |
| >18 | 196.73 ± 10.61 | 202.27 ± 16.42 | 257.21 ± 10.14 | 0.0369 | 5.00 ± 0.48 | 5.10 ± 0.73 | 5.67 ± 0.89 | 0.5725 | 0.10 ± 0.05 | 0.12 ± 0.08 | 0.17 ± 0.05 | 0.5417 |

†logarithm-transformed to adjust the distribution ※The model is adjusted for age, sex, personal income, education, smoking status and alcohol drinking *The statistical significance of a linear trend was tested by including the median of each category as a continuous variable in the regression model.

## 4 Discussion

Because Cr concentration or Cr(VI) concentration in environmental medias is fairly high in exposed areas, people living there are generally at high risk to be exposed to Cr(VI). Since the 1970s, villagers in the exposed areas stopped drinking groundwater thanks to the government's water improvement project, but still use the groundwater to irrigate fields and do some washing. Moreover, they would contact the high Cr concentration soil with their hands and skin when cultivating. Thus, villagers in exposed areas may contact Cr(VI) through cutaneous or hand-to-mouth route. [5] The Cr in air provides a respiration way for exposed villagers to contact higher Cr concentration. [8] Therefore, through all exposure pathways, people living in the Cr(VI)-exposed areas may have a higher risk to be exposed to Cr compared to those in unexposed area.

In this study, our results indicate that adjusted for possible confounders, people living in Cr(VI)-exposed areas have higher lipids and DNA damage levels than those in unexposed areas. In addition, Cr(VI) exposure affects the antioxidant system, such as activation or damaging the antioxidant system.[17 24] Besides significant differences between the exposed and unexposed group, additional factors affect the results, such as sex, age, smoking status and alcohol drinking. Moreover, longer residence in exposed areas may increase the health risk.

Many studies have shown a significant increase of MDA in trivalent chromium [Cr(III)] exposed workers and populations compared to unexposed groups, [25 26] and Cr(VI) exposed workers also have elevated MDA levels compared to unexposed workers. [27] In our study, we find that the MDA concentration of the exposed group is significantly higher than that in the unexposed group adjusted for sex and age or even in the full model. The evaluated MDA concentration indicates increased rate of oxidative stress levels in the lipid in exposed populations. However, results of the stratified analysis show that the MDA concentration is not significantly affected by exposure to Cr(VI) in the subgroup that smokes or consumes alcohol. On the other hand, exposure to Cr(VI) still affects the non-smoking participants and those who do not consume alcohol. Many studies have shown that adjusted for potential confounders, smoking and alcohol drinking can elevate the concentration of MDA in both animal models and in humans.[28 29] However, we do not find significant association either between smoking and MDA concentration or between alcohol drinking and MDA concentration. In our view, this lack of correlation could be mainly due to the strong influence of Cr(VI), which covers the effect that smoking and alcohol drinking have on the MDA concentration. There have been some discrepancies in the concentration of MDA in gender in previous studies. In Cr(III)-exposed populations, the MDA concentration is higher in

females than that in males.[26] However, in normal populations, concentration of MDA is lower in females than that in males, [30] which is consistent with our results.

ROS form when Cr(VI) reduces to a lower oxidation state, and the free radicals may attack DNA thereby disrupting cellular functions and integrity.[19] Thus DNA damage produces alterations in DNA, strand breaks and DNA-protein crosslinks. 8-OHdG is a major oxidative adducts formed by radicals inducing damage to DNA.[31] As a biomarker of oxidative DNA damage, 8-OHdG levels directly reflect the average rate of oxidative DNA damage.[32] Daily cumulative Cr(VI) exposure has a significant correlation with urinary 8-OHdG levels adjusted for covariates in workers.[27 31 33-35]

In our study, concentration of urine 8-OHdG in the exposed group is significantly higher than in the unexposed group (Table 3). This is consistent with previous studies focusing on occupational exposure, which indicates that Cr(VI) exposure induces the formation of ROS and causes oxidative tissue and DNA damage.[36] In its turn, oxidative DNA damage can lead to consequences including cell death, mutation, and malignant transformation.[37] Some studies have shown that the concentration of 8-OHdG mainly correlates with the Cr(VI) concentration in the air.[31 34] Therefore, higher concentration of urinary 8-OHdG in the exposed group may be on account of higher air Cr levels. However, this relation needs further research and evidence.

In the stratified analysis, we find that the level of 8-OHdG regulated with urinary Cre in the elderly group is higher than in the younger group. In the regression model, there is a positive correlation between age and concentration of urine 8-OHdG. A study has shown that a highly significant rise in DNA damage level can be observed in leukocyte DNA in the elderly population (mean age 67 years) and middle age group (mean age 50 years) in comparison with adults (mean age 31 years).[38] These findings are consistent with ours. The reason that DNA damage increases with age may be a deficiency in the ability to remove the damage or the intensification of processes responsible for the damage formation, or both.[38] Some other factors may have effects on the concentration of 8-OHdG, such as smoking status and alcohol drinking. A positive correlation between the 8-OHdG levels and smoking status has been observed, and the 8-OHdG concentration in urinary samples of smokers is 50% higher as compared to nonsmokers.[39 40] Other studies provide evidence that ethanol can induce oxidative DNA damage in human peripheral lymphocytes in vitro and signs of increased oxidative damage compared to the non-drinking people.[41 42] In this study, we find that in both exposed and unexposed group, smokers or drinkers exhibited a higher concentration of 8-OHdG than non-smokers or non-drinkers. This finding is also consistent with previous studies.

In response to oxidative stress and lipid peroxidation, the antioxidant mechanisms are activated. We selected GSH-Px, SOD and CAT as the three parameters to assess the antioxidant mechanisms in response to Cr(VI). Many researchers have shown that the activity of SOD is higher in Cr(VI) occupational exposed group or Cr(III) exposed populations, [25 26 43] while some have shown that the activity of SOD and GSH-Px is decreased in the Cr(VI) occupational-exposed group.[27] GSH-Px catalyzes the reduction of hydrogen peroxide to water and of organic hydro peroxides to less toxicity by using reduced glutathione, and the by-product, oxidized glutathione, is converted to reduced glutathione via the action of glutathione reductase using Nicotinamide Adenine Dinucleotide Phosphate (NADPH) as the electron donor.[44 45] An experiment on cells mentions that GSH-Px and CAT act in a compensatory manner to overcome oxidative stress. CAT accomplishes the basic defense at the late stages of cell growth, and GSH-Px has much higher affinity to $H_2O_2$ than catalase at low substrate concentration.[46]

In our study, we find that the activity of CAT and GSH-Px is higher in the exposed group than that in the unexposed group, which means the antioxidant system is activated. However, the activity of SOD is found to have decreased in the exposed group. Antioxidants play a protective role against free radical induced damage. Therefore, their induction can be understood as a response to oxidative stress. However, if the exposure persists, the antioxidant function can be damaged during or after the exposure.[13 27 46] A decrease in the serum activity of SOD may be a sign of the impairment of the antioxidant system in this study. If the antioxidant systems are not able to reduce the ROS produced, then oxidative stress and oxidative damage may occur, leading to organism disorder, even in some cases to diseases.[17]

In the stratified analysis, we find that Cr(VI) exposure and smoking have an effect modification on GSH-Px activity, and Cr(VI) exposure and alcohol drinking also have an effect modification on GSH-Px activity. In the exposed group, the GSH-Px activity of nonsmokers and nondrinkers is higher than smokers and drinkers. However, in the unexposed group, the GSH-Px activity of nonsmokers and nondrinkers is lower than smokers and drinkers. Animal experiments have shown that alcohol consumption is able to increase oxidative stress, with a decrease in GSH-Px-1 activity and increase of GSH activities. [45 47] In vivo and in vitro experiments, exposure to cigarette smoke could increase intercellular ROS and oxidative stress, and total glutathione decreases dramatically.[48 49] In the unexposed group, smoke and alcohol use may cause the activity of GSH-Px elevating. However, in the exposed group, along with the exposure, the decrease of the activity of GSH-Px may be due to the damage of the antioxidant system. We present participants' disease status (shown in the Appendix C) and conduct stratified analysis according to disease status (shown in the Appendix D), however, we do not see any effects on the primary results.

In the analysis of relationship between length of residence and oxidative levels, we find that urine 8-OHdG concentration and the serum CAT activity have dose-response relationship with residence years in the subgroup of first exposure under 18 years old, and the serum GSH-Px activity in the subgroup of first exposure over 18 years old has a positive correlation with length of residence. These results may indicate that longer exposure to Cr(VI) can aggravate DNA damage and activate antioxidant response. In vitro experiment, cells exposed to Cr(VI) can activate and impair the antioxidant system with the increase of exposure time.[46] In some epidemiology studies, researchers found that different length of residence may have an effect on the oxidative stress levels exposed to heavy metal in women immigrants.[50] A cohort study of chromate production workers indicates that exposure length is an important explanatory variable to the increase of lung cancer risk.[51] Our study reveals that long-term exposure to Cr(VI) can continuously increase the health risk.

The main limitation of our study is that individual-exposure data is not obtained. This may lead to the major problem which is that we could not relate internal exposure to oxidative parameters. We will keep working on this project, trying to get more data to give a further clarification of the relationship between the health effects and Cr(VI) contamination. In addition, homogeneity of the exposed and unexposed groups is not satisfactory. For this reason, we used a multiple regression model and stratified analysis to adjust for possible confounders. Also we did not take people's nutritional status into consideration, mainly because participants are all living in the rural areas of Jinzhou, Liaoning province , whose diet and living habits are basically the same.

In conclusion, our research demonstrates that people living around the ferroalloy factory are at higher health risk. After adjusting for potential confounders, results show elevated oxidative stress and oxidative damage in the population exposed to Cr(VI) compared to the unexposed population. Moreover, the effect modification presented in the stratified analysis may indicate that the combination of both Cr(VI) and alcohol or both Cr(VI) and smoking may cause damage to antioxidant system. In addition, longer residence exposed to Cr(VI) would increase people's oxidative levels.

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**Competing interests**

The authors declare that they have no competing interests.

**Authors' contributions**

Jing Xu analyzed and interpreted the data and was a major contributor in writing the manuscript. Meiduo Zhao, Lu Pei and Ruiming Zhang participated in the field work and experiment work. Xiaolin Liu, Lanping Wei prepared all the things relating to the field work. Mingan Yang was in charge of polishing the manuscript and conducting some statistical analysis. Qun Xu designed the study and arranged the field work. All authors read and approved the final manuscript.

**Data sharing statement**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Figure Legend**

Figure 1 shows the position of six villages where we conduct a survey, with A1 village, A2 village and A3 village as exposed areas, and B1 village, B2 village, and B3 village as unexposed areas. All the exposed villages were along the contaminated river, less than 10 kilometers away from the factory. The unexposed villages were at least 50 kilometers away from the factory. This map was generated with the ArcGIS Online base map which was publicly available and produced by the ArcGIS 10.2 software.

**References**

1. Kim HS, Kim YJ, Seo YR. An Overview of Carcinogenic Heavy Metal: Molecular Toxicity Mechanism and Prevention. *Journal of Cancer Prevention* 2015;20(4):232-40. doi: 10.15430/jcp.2015.20.4.232
2. Qu X, Ren Z, Zhang M, et al. Sediment heavy metals and benthic diversities in Hun-Tai River, northeast of China. *Environ Sci Pollut Res Int* 2017 doi: 10.1007/s11356-017-8642-0 [published Online First: 2017/03/12]
3. Mazurek R, Kowalska J, Gasiorek M, et al. Assessment of heavy metals contamination in surface layers of Roztocze National Park forest soils (SE Poland) by indices of pollution. *Chemosphere* 2017;168:839-50. doi: 10.1016/j.chemosphere.2016.10.126 [published Online First: 2016/11/11]
4. Navarrete IA, Gabiana CC, Dumo JR, et al. Heavy metal concentrations in soils and vegetation in urban areas of Quezon City, Philippines. *Environmental monitoring and assessment* 2017;189(4):145. doi: 10.1007/s10661-017-5849-y [published Online First: 2017/03/08]
5. OLIVER MA. Soil and human health a review. *European Journal of Soil Science* 1997; 48:573-92.
6. Toxicguide for chromium. *US Department of Health and Human Services Public Health Service Agency for Toxic Substances and Disease Registry* 2012
7. Linos A, Petralias A, Christophi CA, et al. Oral ingestion of hexavalent chromium through drinking water and cancer mortality in an industrial area of Greece--an ecological study. *Environ Health* 2011;10:50. doi: 10.1186/1476-069X-10-50
8. Kerger BD, Butler WJ, Paustenbach DJ, et al. Cancer mortality in chinese populations surrounding an alloy plant with chromium smelting operations. *J Toxicol Environ Health A* 2009;72(5):329-44. doi: 10.1080/15287390802529898
9. Chromium, nickel and welding. *IARC monographs on the evaluation of carcinogenic risks to humans* 1990;49:1-648. [published Online First: 1990/01/01]
10. Welling R, Beaumont JJ, Petersen SJ, et al. Chromium VI and stomach cancer: a meta-analysis of the current

epidemiological evidence. *Occup Environ Med* 2015;72(2):151-9. doi: 10.1136/oemed-2014-102178

11. Beaumont JJ, Sedman RM, Reynolds SD, et al. Cancer mortality in a Chinese population exposed to hexavalent chromium in drinking water. *Epidemiology* 2008;19(1):12-23. doi: 10.1097/EDE.0b013e31815cea4c
12. M. Valko HM, M.T.D. Cronin. Metals, Toxicity and Oxidative Stress. *Current Medicinal Chemistry* 2005;12:1161-208.
13. Patlolla AK, Barnes C, Yedjou C, et al. Oxidative stress, DNA damage, and antioxidant enzyme activity induced by hexavalent chromium in Sprague-Dawley rats. *Environmental toxicology* 2009;24(1):66-73. doi: 10.1002/tox.20395 [published Online First: 2008/05/30]
14. Jomova K, Valko M. Advances in metal-induced oxidative stress and human disease. *Toxicology* 2011;283(2-3):65-87. doi: 10.1016/j.tox.2011.03.001
15. Sharma B, Singh S, Siddiqi NJ. Biomedical implications of heavy metals induced imbalances in redox systems. *Biomed Res Int* 2014;2014:640754. doi: 10.1155/2014/640754
16. Dalle-Donne I, Rossi R, Colombo R, et al. Biomarkers of oxidative damage in human disease. *Clinical chemistry* 2006;52(4):601-23. doi: 10.1373/clinchem.2005.061408 [published Online First: 2006/02/18]
17. Sharma N. Free Radicals, Antioxidants and Disease. *Biology and Medicine* 2014;06(03) doi: 10.4172/0974-8369.1000214
18. Kaur R, Kaur J, Mahajan J, et al. Oxidative stress--implications, source and its prevention. *Environ Sci Pollut Res Int* 2014;21(3):1599-613. doi: 10.1007/s11356-013-2251-3 [published Online First: 2013/10/31]
19. Bagchi D, Vuchetich PJ, Bagchi M, et al. Induction of oxidative stress by chronic administration of sodium dichromate [chromium VI] and cadmium chloride [cadmium II] to rats. *Free radical biology & medicine* 1997;22(3):471-8. [published Online First: 1997/01/01]
20. EDWIN N.FRANKEL WEV. Formation of Malonaldehyde from Lipid Oxidation Products. *Biochmicu et Biophvsicu Acta* 1983;754:264-70.
21. GB 7467-87. Water quality-Determination of chrommium( Ⅵ )-1.5 Diphenylcarbohydrazide spectrophotometric method. *Ministry of Environmental Protection of the People's Republic of China* 1987
22. HJ 491-2009. Soil quality-Determination of total chromium -Flame atomic absorption spectrometry. *Ministry of Environmental Protection of the People's Republic of China* 2009
23. HJ 777-2015. Ambient air and waste gas from stationary sources emission-Determination of metal elements in ambient particle matter-Inductively coupled plasma optical emission spectrometry. *Ministry of Environmental Protection of the People's Republic of China* 2015
24. Junaid M, Hashmi MZ, Malik RN, et al. Toxicity and oxidative stress induced by chromium in workers exposed from different occupational settings around the globe: A review. *Environ Sci Pollut Res Int* 2016 doi: 10.1007/s11356-016-7463-x
25. Ambreen K, Khan FH, Bhadauria S, et al. Genotoxicity and oxidative stress in chromium-exposed tannery workers in North India. *Toxicol Ind Health* 2014;30(5):405-14. doi: 10.1177/0748233712457447
26. Khan FH, Ambreen K, Fatima G, et al. Assessment of health risks with reference to oxidative stress and DNA damage in chromium exposed population. *Sci Total Environ* 2012;430:68-74. doi: 10.1016/j.scitotenv.2012.04.063
27. Wang TC, Song YS, Wang H, et al. Oxidative DNA damage and global DNA hypomethylation are related to folate deficiency in chromate manufacturing workers. *J Hazard Mater* 2012;213-214:440-6. doi: 10.1016/j.jhazmat.2012.02.024
28. Megson IL, Haw SJ, Newby DE, et al. Association between exposure to environmental tobacco smoke and biomarkers of oxidative stress among patients hospitalised with acute myocardial infarction. *PLoS One* 2013;8(12):e81209. doi: 10.1371/journal.pone.0081209 [published Online First: 2013/12/18]

29. McCaskill ML, Kharbanda KK, Tuma DJ, et al. Hybrid malondialdehyde and acetaldehyde protein adducts form in the lungs of mice exposed to alcohol and cigarette smoke. *Alcoholism, clinical and experimental research* 2011;35(6):1106-13. doi: 10.1111/j.1530-0277.2011.01443.x [published Online First: 2011/03/25]

30. Bloomer RJ, Fisher-Wellman KH. Blood oxidative stress biomarkers: influence of sex, exercise training status, and dietary intake. *Gender medicine* 2008;5(3):218-28. doi: 10.1016/j.genm.2008.07.002 [published Online First: 2008/08/30]

31. Kuo HW, Chang SF, Wu KY, et al. Chromium (VI) induced oxidative damage to DNA: increase of urinary 8-hydroxydeoxyguanosine concentrations (8-OHdG) among electroplating workers. *Occup Environ Med* 2003;60(8):590-4. [published Online First: 2003/07/29]

32. Floyd RA. The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis* 1990;11(9):1447-50. [published Online First: 1990/09/01]

33. Pan CH, Jeng HA, Lai CH. Biomarkers of oxidative stress in electroplating workers exposed to hexavalent chromium. *Journal of exposure science & environmental epidemiology* 2017 doi: 10.1038/jes.2016.85 [published Online First: 2017/01/26]

34. Li P, Gu Y, Yu S, et al. Assessing the suitability of 8-OHdG and micronuclei as genotoxic biomarkers in chromate-exposed workers: a cross-sectional study. *BMJ Open* 2014;4(10):e005979. doi: 10.1136/bmjopen-2014-005979

35. Ni W, Huang Y, Wang X, et al. Associations of neonatal lead, cadmium, chromium and nickel co-exposure with DNA oxidative damage in an electronic waste recycling town. *Sci Total Environ* 2014;472:354-62. doi: 10.1016/j.scitotenv.2013.11.032

36. Bagchi D, Hassoun EA, Bagchi M, et al. Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production, and generation of reactive oxygen species in Sprague-Dawley rats. *Comparative biochemistry and physiology Part C, Pharmacology, toxicology & endocrinology* 1995;110(2):177-87. [published Online First: 1995/02/01]

37. Wise SS, Holmes AL, Wise JP, Sr. Hexavalent chromium-induced DNA damage and repair mechanisms. *Reviews on environmental health* 2008;23(1):39-57. [published Online First: 2008/06/19]

38. Olinski R, Siomek A, Rozalski R, et al. Oxidative damage to DNA and antioxidant status in aging and age-related diseases. *Acta biochimica Polonica* 2007;54(1):11-26. [published Online First: 2007/01/16]

39. Kasai H. Analysis of a form of oxidative DNA damage,8-hydroxy-2X-deoxyguanosine, as a marker of cellular oxidative stress during carcinogenesis. *Mutation Research* 1997 387:147-63.

40. Steffen Loft KV, Marianne Ewertz,Anne Tj0nneland, Kim Overvad,Henrik Enghusen Poulsen. Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Cardnogeoesis* 1992;13 (12):2241-47.

41. Yan Y, Yang JY, Mou YH, et al. Possible metabolic pathways of ethanol responsible for oxidative DNA damage in human peripheral lymphocytes. *Alcoholism, clinical and experimental research* 2011;35(1):1-9. doi: 10.1111/j.1530-0277.2010.01316.x [published Online First: 2010/10/21]

42. Rendon-Ramirez A, Cortes-Couto M, Martinez-Rizo AB, et al. Oxidative damage in young alcohol drinkers: A preliminary study. *Alcohol (Fayetteville, NY)* 2013;47(7):501-4. doi: 10.1016/j.alcohol.2013.08.002 [published Online First: 2013/10/02]

43. Junaid M, Hashmi MZ, Malik RN. Evaluating levels and health risk of heavy metals in exposed workers from surgical instrument manufacturing industries of Sialkot, Pakistan. *Environ Sci Pollut Res Int* 2016 doi: 10.1007/s11356-016-6849-0

44. Gromadzinska J, Wasowicz W, Sklodowska M, et al. The influence of atmospheric chromium on selenium content and glutathione peroxidase activity in blood of tannery workers. *Environ Health Perspect* 1996;104(12):1312-6. [published Online First: 1996/12/01]

45. Bailey SM, Patel VB, Young TA, et al. Chronic ethanol consumption alters the glutathione/glutathione

peroxidase-1 system and protein oxidation status in rat liver. *Alcoholism, clinical and experimental research* 2001;25(5):726-33. [published Online First: 2001/05/24]

46. Asatiani N, Kartvelishvili T, Abuladze M, et al. Chromium (VI) can activate and impair antioxidant defense system. *Biological trace element research* 2011;142(3):388-97. doi: 10.1007/s12011-010-8806-y [published Online First: 2010/09/03]
47. Kaphalia L, Boroumand N, Hyunsu J, et al. Ethanol metabolism, oxidative stress, and endoplasmic reticulum stress responses in the lungs of hepatic alcohol dehydrogenase deficient deer mice after chronic ethanol feeding. *Toxicol Appl Pharmacol* 2014;277(2):109-17. doi: 10.1016/j.taap.2014.02.018 [published Online First: 2014/03/15]
48. Colombo G, Dalle-Donne I, Orioli M, et al. Oxidative damage in human gingival fibroblasts exposed to cigarette smoke. *Free radical biology & medicine* 2012;52(9):1584-96. doi: 10.1016/j.freeradbiomed.2012.02.030 [published Online First: 2012/03/06]
49. Ignatowicz E, Wozniak A, Kulza M, et al. Exposure to alcohol and tobacco smoke causes oxidative stress in rats. *Pharmacological reports : PR* 2013;65(4):906-13. [published Online First: 2013/10/23]
50. Wu WT, Wu CC, Lin YJ, et al. Changing blood lead levels and oxidative stress with duration of residence among Taiwan immigrants. *Journal of immigrant and minority health* 2013;15(6):1048-56. doi: 10.1007/s10903-013-9820-x [published Online First: 2013/04/06]
51. Gibb H, Hoffman H, Haver C. Biologic Implications from an Epidemiologic Study of Chromate Production Workers. The Open Epidemiology Journal2011.

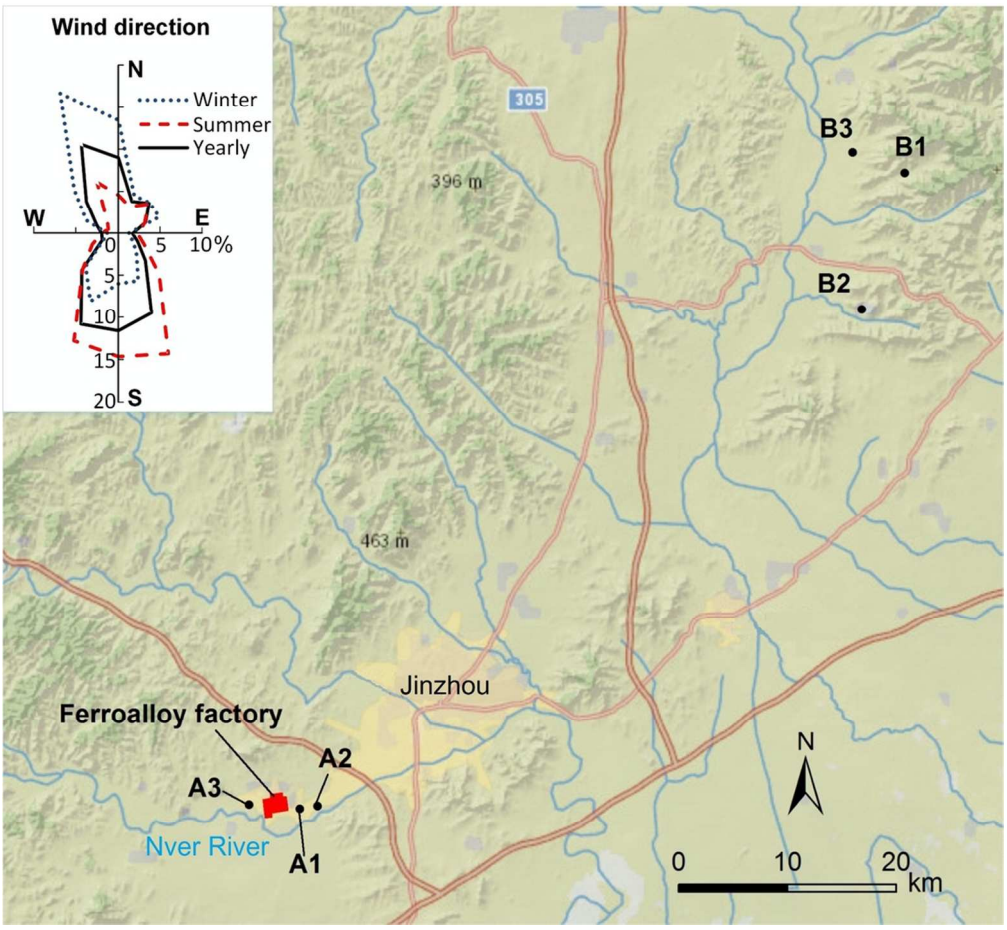


Figure 1.Locations of Exposed Areas, Unexposed Areas and Ferroalloy Factory

97x89mm (300 x 300 DPI)

# Appendix

**Appendix A. The estimate and p value of all variables in the regression Model 1**

| | | MDA（nmol/ml) | | SOD(U/ml) | | CAT(U/mL) | | GSH-Px(U/mL) | | Lg(8-OHdG)(μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β | p value | β | p value | β | p value | β | p value | β | p value |
| Age | | | | | | | | | | | |
| | ＜60 | ref | | ref | | ref | | ref | | ref | |
| | ⩾60 | -0.14 | 0.089 | 1.65 | 0.160 | -0.255 | 0.186 | 6.30 | 0.273 | 0.12 | ＜0.001 |
| Sex | | | | | | | | | | | |
| | male | ref | | ref | | ref | | ref | | ref | |
| | female | -0.22 | 0.014 | 1.96 | 0.135 | -0.71 | 0.001 | -6.62 | 0.298 | 0.06 | 0.086 |
| Exposure status | | | | | | | | | | | |
| | | ref | | ref | | ref | | ref | | ref | |
| unexposed | | | | | | | | | | | |
| | exposed | 0.33 | <0.001 | -14.92 | <0.001 | 1.55 | <0.001 | 43.69 | <0.001 | 0.08 | 0.012 |

**Appendix B. The estimate and p value of all variables in the regression of Model 2**

| | MDA (nmol/m) | | SOD (U/ml) | | CAT (U/mL) | | GSH-Px (U/mL) | | Lg(8-OHdG) (μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | β | p value | β | p value | β | p value | β | p value | β | p value |
| Age | | | | | | | | | | |
| < 60 | ref | | ref | | ref | | ref | | ref | |
| ≥60 | -0.11 | 0.208 | 2.01 | 0.108 | -0.279 | 0.175 | 3.96 | 0.517 | 0.11 | 0.002 |
| Sex | | | | | | | | | | |
| male | ref | | ref | | ref | | ref | | ref | |
| female | -0.18 | 0.111 | 2.43 | 0.133 | -0.95 | <0.001 | -13.97 | 0.076 | 0.10 | 0.025 |
| Smoking status | | | | | | | | | | |
| no | ref | | ref | | ref | | ref | | ref | |
| yes | -0.01 | 0.895 | -0.10 | 0.948 | -0.08 | 0.758 | -14.61 | 0.049 | 0.01 | 0.791 |
| Alcohol drinking | | | | | | | | | | |
| no | ref | | ref | | ref | | ref | | ref | |
| yes | 0.08 | 0.552 | 1.19 | 0.537 | -0.47 | 0.136 | -2.76 | 0.771 | 0.08 | 0.170 |
| Education | | | | | | | | | | |
| Primary or lower | ref | | ref | | ref | | ref | | ref | |
| Middle or higher | 0.02 | 0.825 | 0.72 | 0.568 | -0.27 | 0.189 | -11.16 | 0.071 | -0.018 | 0.617 |
| Personal income | | | | | | | | | | |
| <2000 | ref | | ref | | ref | | ref | | ref | |
| 2000-5000 | 0.15 | 0.150 | -0.36 | 0.810 | 0.20 | 0.424 | 2.02 | 0.781 | -0.017 | 0.693 |
| >5000 | 0.12 | 0.211 | 0.11 | 0.940 | 0.20 | 0.4.5 | -2.84 | 0.687 | -0.032 | 0.430 |
| Exposure status | | | | | | | | | | |
| unexposed | ref | | ref | | ref | | ref | | ref | |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| exposed | 0.32 | <0.001 | -14.93 | <0.001 | 1.60 | <0.001 | 45.66 | <0.001 | 0.093 | 0.0075 |

**Appendix C. The prevalence of diabetes and hypertension in exposed and unexposed areas**

| Variables | All | Hexavalent chromium | | *P* value[2] |
|---|---|---|---|---|
| | (n=626) | Exposure | None-exposure | |
| No. of subjects | 620 | 315 | 305 | |
| Diabetes [n (%)] | | | | 0.0284 |
| No | 545 ( 87.90 ) | 268 ( 85.08 ) | 277 ( 90.82 ) | |
| Yes | 75 ( 12.10 ) | 47 ( 14.92 ) | 28 ( 9.18 ) | |
| Hypertension [n (%)] | | | | 0.5388 |
| No | 348 ( 56.13 ) | 173 ( 54.92 ) | 175( 57.38 ) | |
| Yes | 272 ( 43.87 ) | 142 ( 45.08 ) | 130 ( 42.62 ) | |

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**Appendix D. Average levels of oxidative parameters according to exposure status in different disease status**

| | | MDA(nmol/ml) | | SOD(U/ml) | | CAT(U/mL) | | GSHPx(U/mL) | | Lg(8-OHdG)(μg/mmol-Cre) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed | Exposed | Unexposed |
| Diabetes | no | 3.59±0.07** | 3.28±0.06 | 54.05±1.04** | 68.68±0.96 | 4.81±0.19** | 3.33±0.16 | 195.86±5.11 | 149.10±4.71 | 0.13±0.03* | 0.04±0.03 |
| | | (260) | (275) | ( 263 ) | (275) | (265) | (275) | (254) | (273) | (238) | (234) |
| | yes | 3.86±0.21 | 3.50±0.28 | 51.52±3.32** | 69.38±4.40 | 5.52±0.59* | 3.12±0.78 | 191.08±15.55** | 173.46±20.88 | 0.08±0.08 | -0.12±0.11 |
| | | (46) | (28) | (47) | (27) | (47) | (28) | (45) | (28) | (42) | (25) |
| Hypertension | no | 3.64±0.09** | 3.25±0.08 | 53.88±1.25** | 69.11±1.17 | 5.07±0.21** | 3.36±0.20 | 192.43±6.50** | 151.13±5.98 | 0.05±0.04 | 0.02±0.04 |
| | | (166) | (173) | (171) | (174) | (171) | (173) | (162) | (172) | (148) | (144) |
| | yes | 3.62±0.10* | 3.34±0.10 | 53.21±1.57** | 67.71±1.57 | 4.78±0.26** | 3.25±0.26 | 199.45±7.28** | 151.98±7.32 | 0.16±0.04* | 0.01±0.04 |
| | | (140) | (130) | (139) | (130) | (141) | (130) | (137) | (129) | (132) | (115) |