BMJ Open Oxidative stress and DNA damage in a long-term hexavalent chromium-exposed population in North China: a cross-sectional study

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ABSTRACT

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

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

Participants The study included 319 participants exposed to Cr(VI) and 307 unexposed participants, with 447 women and 179 men. These participants met the following criteria: (1) living in the areas for more than 10 years; (2) age older than 18 years; and (3) 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 those in the unexposed group. However, serum SOD activity was significantly lower in the exposed group, compared with that in the unexposed group ($p<0.001$). Cr(VI) exposure and smoking have an interaction effect on GSH-Px activity ($p<0.05$). Cr(VI) exposure and alcohol drinking also have an interaction effect on GSH-Px activity ($p<0.05$). Longer residence in the exposed areas increased the oxidative levels ($p<0.05$).

Conclusions The findings of this study showed elevated oxidative stress and DNA damage in people exposed to Cr(VI).

INTRODUCTION

Hexavalent chromium (Cr(VI)) compounds are commonly found in industrial settings

Strengths and limitations of this study

- 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.
- Health survey and environmental surveillance for hexavalent chromium were conducted concurrently in previously environmentally polluted areas.
- We collected individual demographic characteristics and lifestyle data to minimise possible confounding in the analysis.
- The main limitation is that individual exposure data are not obtained.
- In addition, demographic homogeneity of the exposed and unexposed groups was not so satisfactory.

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

According to the International Agency for Research on Cancer monographs on the evaluation of carcinogenic risks to humans, Cr(VI) is considered a human carcinogen.⁷⁻¹⁰ Based on numerous studies in occupational epidemiology, inhalation of Cr(VI) correlated with increased risk for lung cancer.⁹ Numerous epidemiological studies also reported an increased risk of cancer morbidity, especially

for gastrointestinal cancer, in populations exposed to Cr(VI).^{7 11} For these reasons, Cr(VI) contamination may pose a serious threat to population health.

Toxicity and carcinogenicity of Cr(VI) are 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 substance that is the best marker for oxidative damage in an organism is 8-hydroxy-2'-deoxyguanosine (8-OHdG).¹⁴ Reduction of the extra ROS can be achieved through enzymatic and non-enzymatic reactions. Oxidative stress results from an imbalance between the production of free radicals and the antioxidant defence 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 the depletion of the antioxidant defence elements, subsequently causing lipid peroxidation.¹⁹ Lipid peroxidation has been suggested to play a key role in many biological processes, and malondialdehyde (MDA) has long been used as a marker for secondary products of lipid peroxidation.²⁰

Due to industrial expansion in the mid-20th century, the western suburban areas of Jinzhou city in Liaoning province in north-east 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) by respiratory route, and by 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 the water has been polluted by the ferroalloy factory. Previous studies have shown that the highest Cr concentration in well water of this area was 20 mg/L.¹¹ A ferroalloy factory was established here 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. Therefore, this study mainly aims to investigate whether environmental exposure to Cr(VI) can induce changes in oxidative stress and oxidative damage.

MATERIALS AND METHODS

Study design and population

We conducted a cross-sectional study in the villages of Jinzhou city located in Liaoning province in north-east

China to evaluate changes in the levels of oxidative stress and oxidative damage caused by Cr(VI). We enrolled 626 participants, 447 women and 179 men, in the study who met the following criteria: (1) living in the areas for more than 10 years; (2) age older than 18 years; and (3) 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 the exposed areas, and B1 village, B2 village and B3 village as the unexposed areas. **Figure 1** shows a map of the study areas, which was generated using the ArcGIS Online basemap publicly available and produced by the ArcGIS V.10.2 software. All the exposed villages were along the contaminated river, less than 10 km away from the ferroalloy factory, with high Cr levels in the environment. The unexposed villages were at least 50 km away from the factory, with relatively low Cr levels in the environment (**figure 1**, **table 1**). All enrolled individuals signed the informed consent forms.

Questionnaire survey

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

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 min 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 the remaining samples were stored at -80°C for later analysis.

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

The serum concentration of malondialdehyde (MDA) and the serum activities of catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were determined with Malondialdehyde (MDA) Assay Kit (thiobarbituric acid method), Catalase (CAT) Assay Kit (visible light), Total Superoxide Dismutase (T-SOD) Assay Kit (hydroxylamine method) and Glutathione Peroxidase (GSH-Px) Assay Kit (colourimetric method), which were supplied by Nanjing Jiancheng Bioengineering Institute (China).

Figure 1 Position of six villages where the survey was conducted, 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 km away from the factory. The unexposed villages were at least 50 km away from the factory. This map was generated using the ArcGIS Online basemap, which was publicly available and produced by the ArcGIS V.10.2 software.

Urinary 8-OHdG and urinary creatinine measurements

Urine concentration of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was determined with an ELISA kit (JaICA, Japan, 8-OHdG ELISA kit). To minimise the influence of urine density difference among participants, the 8-OHdG concentration was regulated with urine creatinine (Cre), which was determined by ELISA using a commercial kit from Roche Pharmaceutical (Switzerland).

Collection and testing of environmental media samples

Environmental media samples were collected in the studied area at the same time as the survey was conducted.

We collected groundwater samples from 7 m or 8 m deep wells in the yards of the participant's house and soil samples from the field surface. Air samples were collected 24 hours a day for 5 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. The concentration of Cr(VI) in the groundwater was determined by diphenylcarbazide spectrophotometric method with a detection limit of 0.004 mg/L.²¹ If the detection result was lower than the limit, half of the detection limit was used in the statistical analysis. The total Cr level of soil

Table 1 Chromium levels in varied samples collected from the study areas in 2016

Samples	Exposed areas		Unexposed areas		P values*
	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

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

†Hexavalent chromium.

and air was determined by atomic absorption spectrophotometry and inductively coupled plasma optical emission spectrometry.^{22 23}

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 the 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 strictly follow the protocol and instructions of the kit in conducting the analysis. Absorbance for each specimen was measured three times, using the average as its final value. If its fluctuation was more than 50% among the three absorbance measures, the analysis would be reconducted. Testing of the environmental samples was strictly conducted according to the protocol.

Statistical analysis

EpiData V.3.1 software was used for input of original data collected from questionnaires with double-entry, checking for logic errors to ensure accuracy. Summary statistics were provided for both categorical (proportion) and continuous variables. Unpaired t-test was used to compare two mean values and χ^2 test was used to compare categorical variables. If the data did not meet the normal distribution, Wilcoxon rank-sum test was used.

Subsequently, multiple general linear regression analysis was performed to analyse 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 the multivariate regression analysis because these two variables had less than 10% of cases in a group. All the expected and observed numbers with the variables used in the multivariate regression model had more than 5 in each cell of the two-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 conducted using the SAS V.9.4 software.

Patients and public involvement

Patients and the public were not involved in this study.

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 the maximum concentration of Cr(VI) in the groundwater samples in the exposed areas reached 2.5 mg/L, with a statistically significant 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 2 presents the demographic characteristics of the 626 participants living in the exposed and unexposed areas, 319 in the exposed villages and 307 in the unexposed. **Table 2** shows there is no significant difference in occupation, marital status or personal income between the 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 group.

The results of the 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 with 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, the results remained statistically significant 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 in the unexposed group, adjusted for sex and age (**table 3**, model 1), which remained significantly lower after further adjusting 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 online supplementary appendix A and appendix B). **Table 4** shows the difference in serum MDA concentration between the exposed and unexposed groups, with analysis for subgroups categorised 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

Table 2 Demographic characteristics of the study participants

Variables	All	Hexavalent chromium		P values*
	(n=626)	Exposure (n=319)	No exposure (n=307)	
Age (years)	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 (¥), 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 χ^2 test was used for categorical variables.

have an interaction effect on GSH-Px activity. Cr(VI) exposure and alcohol drinking also have an interaction effect on GSH-Px activity (table 4).

The length of residence positively associated with the oxidative levels (table 5), with a mean of 45 years and SD 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 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.

DISCUSSION

Because Cr concentration or Cr(VI) concentration in environmental media samples is fairly high in exposed areas, people living in these areas are generally at high risk of being exposed to Cr(VI). Since the 1970s, villagers in the exposed areas have stopped drinking groundwater, thanks to the government's water improvement project, but they still use groundwater to irrigate fields and do some washing. Moreover, they can get exposed to soil with high Cr concentration through their hands and skin

when cultivating. Thus, villagers in exposed areas may get in contact with Cr(VI) through cutaneous or hand-to-mouth route.⁵ Villagers can also get exposed to higher Cr concentrations through respiration.⁸ Therefore, with all these exposure pathways, people living in the Cr(VI)-exposed areas may have a higher risk of being exposed to Cr compared with those in unexposed areas.

The results of this study indicate that, adjusting for possible confounders, people living in Cr(VI)-exposed areas have higher levels of lipid and DNA damage than those in unexposed areas. In addition, Cr(VI) exposure affects the antioxidant system, such as by activating or damaging the antioxidant system.^{17–24} Besides, sex, age, smoking status and alcohol drinking affect the oxidative levels. 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 with unexposed groups,^{25–26} and Cr(VI)-exposed workers also have elevated MDA levels compared with unexposed workers.²⁷ In our study, we find that the MDA concentration of the exposed group

Table 3 Average oxidative parameters according to exposure and no exposure

Parameter	Model	Exposure (Lsmean±SE)	No exposure (Lsmean±SE)	β*	P values
MDA (nmol/mL)	Model 1	3.62±0.06	3.29±0.06	0.33	0.0001
	Model 2	3.65±0.07	3.33±0.06	0.32	0.0001
SOD (U/mL)	Model 1	53.87±0.90	68.80±0.85	-14.92	<0.0001
	Model 2	54.06±1.02	68.99±0.95	-14.93	<0.0001
GSH-Px (U/mL)	Model 1	197.47±4.44	153.77±4.12	43.69	<0.0001
	Model 2	194.99±5.00	149.33±4.64	45.66	<0.0001
CAT (U/mL)	Model 1	4.86±0.15	3.31±0.14	1.55	<0.0001
	Model 2	4.77±0.17	3.17±0.16	1.60	<0.0001
8-OHdG† (ng/μmol-Cre)	Model 1	0.11±0.03	0.02±0.02	0.08	0.0117
	Model 2	0.12±0.03	0.03±0.03	0.09	0.0075

Model 1 is adjusted for sex and age.

Model 2 is adjusted for sex, age, personal income, education, smoking and alcohol use.

P value of every model is less than 0.05.

*Beta coefficient of regression, with the unexposed as the reference.

†Logarithm-transformed for normal distribution.

8-OHdG, 8-hydroxy-2 deoxyguanosine; CAT, catalase; Cre, creatinine; GSH-Px, glutathione peroxidase; Lsmean, least squares mean; MDA, malondialdehyde; SOD, superoxide dismutase.

is significantly higher than that in the unexposed group, adjusting for sex and age or even with the full model. The evaluated MDA concentration indicates increased rate of oxidative stress levels in the lipids of exposed populations. However, the results of the stratified analysis show that 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 adjusting 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 effects 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 women than that in men.²⁶ However, in normal populations, concentration of MDA is lower in women than that in men,³⁰ which is consistent with our results.

ROS form when Cr(VI) reduces to a lower oxidation state, and the free radicals may attack the DNA, thereby disrupting cellular functions and integrity.¹⁹ Thus DNA damage produces alterations in the DNA, strand breaks and DNA-protein crosslinks. 8-OHdG is a major oxidative adduct formed by radicals inducing damage to the 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, the 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 leucocyte 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

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

8-OHdG, 8-hydroxy-2 deoxyguanosine; CAT, catalase; Cre, creatinine; GSH-Px, glutathione peroxidase; Lsmeans, least squares mean; MDA, malondialdehyde; SOD, superoxide dismutase; Lg, base-10 logarithm.

Table 5 Relationship between length of residence and oxidative parameters stratified 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	39–55	≥56	P _{for trend‡}	≤38	39–55	≥56	P _{for trend}	≤38	39–55	≥56	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

*The model is adjusted for age, sex, personal income, education level, smoking status and alcohol drinking.

†Logarithm-transformed to adjust the distribution.

‡The statistical significance of a linear trend was tested by including the median of each category as a continuous variable in the regression model.

8-OHdG, 8-hydroxy-2 deoxyguanosine; CAT, catalase; Cre, creatinine; GSH-Px, glutathione peroxidase; Lsmeans, least squares mean;

is 50% higher as compared with non-smokers.^{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 with the non-drinking people.^{41 42} In this study, we find that in both exposed and unexposed groups, 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 the occupational Cr(VI)-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 occupational Cr(VI)-exposed group.²⁷ GSH-Px catalyses the reduction of hydrogen peroxide to water and of organic hydroperoxides to less toxicity by using reduced glutathione, and the by-product, oxidised glutathione, is converted to reduced glutathione via the action of glutathione reductase using nicotinamide adenine dinucleotide phosphate 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 defence at the late stages of cell growth, and GSH-Px has much higher affinity to H₂O₂ than CAT 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, and even to diseases in some cases.¹⁷

In the stratified analysis, we find that Cr(VI) exposure and smoking have an effect modification on GSH-Px activity, and that Cr(VI) exposure and alcohol drinking also have an effect modification on GSH-Px activity. In the exposed group, the GSH-Px activity of non-smokers and non-drinkers is higher than smokers and drinkers. However, in the unexposed group, the GSH-Px activity of non-smokers and non-drinkers 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-I activity and increase in GSH activities.^{45 47} In 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 elevated activity of GSH-Px. 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 online supplementary appendix C) and conduct stratified analysis according to disease status (shown in online supplementary appendix D); however, we do not see any effects on the primary results.

In the analysis of the relationship between the length of residence and oxidative levels, we find that urine 8-OHdG concentration and serum CAT activity have a dose-response relationship with years of residence 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 the length of residence. These results may indicate that longer exposure to Cr(VI) can aggravate DNA damage and activate antioxidant response. In an in vitro experiment, cells exposed to Cr(VI) can activate and impair the antioxidant system with the increase in exposure time.⁴⁶ In some epidemiology studies, researchers found that different lengths of residence may have an effect on the oxidative stress levels of female immigrants exposed to heavy metal.⁵⁰ A cohort study of chromate production workers indicates that length of exposure 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 are not obtained. This may lead to a major problem, which is that we could not relate the 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, the results show elevated oxidative stress and oxidative damage in the population exposed to Cr(VI) compared with 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 the antioxidant system. In addition, longer residence in areas exposed to Cr(VI) would increase people's oxidative levels.

Contributors JX analysed and interpreted the data and was a major contributor to writing the manuscript. MZ, LP and RZ participated in the field work and

experimental work. XL and LW prepared all the things relating to the field work. MY was in charge of polishing the manuscript and conducting some statistical analyses. QX designed the study and arranged the field work. All authors read and approved the final manuscript.

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